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HEAVY METAL LOADING AND ITS IMPACT ON *Villorita cyprinoides* (HANLEY) ALONG THE ESTUARIES OF SOUTH WEST COAST OF INDIA

THESIS SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

REFERENCE ONLY

IN
FISH & FISHERIES SCIENCE
(MARICULTURE)

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BY

A. PRIYALEKSHMI

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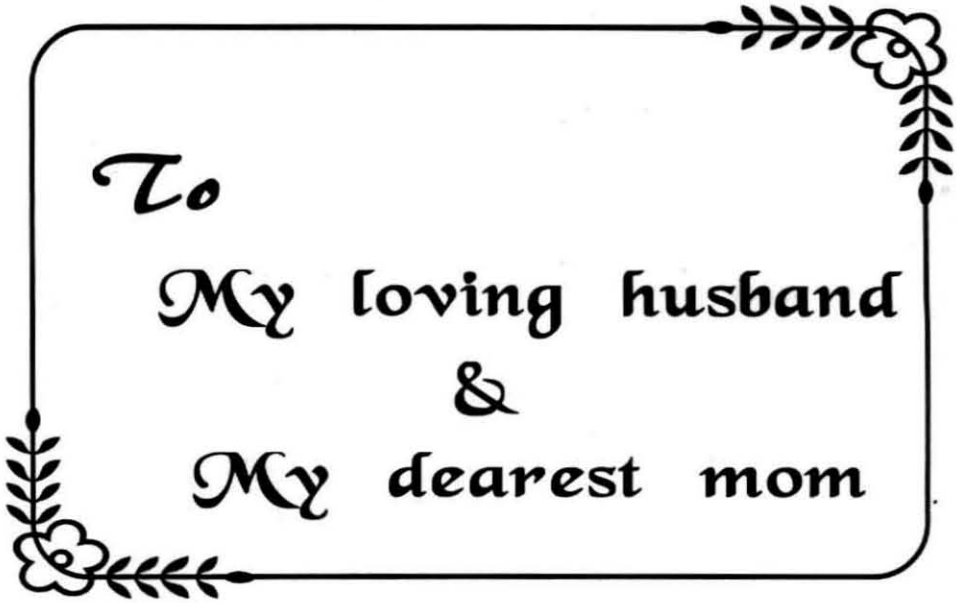
To
My loving husband
&
My dearest mom

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CERTIFICATE

Certified that the thesis entitled **"HEAVY METAL LOADING AND ITS IMPACT ON VILLORITA CYPRINOIDES (HANLEY) ALONG THE ESTUARIES OF SOUTH WEST COAST OF INDIA"** is a record of independent bonafide research work carried out by **A. PRIYA LEKSHMI** during the period of study from November 1996 to July 2001 under our supervision and guidance for the degree of **Doctor of Philosophy (Mariculture)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that this Thesis entitiled "HEAVY METAL LOADING AND ITS IMPACT ON *Villorita cyprinoides* (HANLEY) ALONG THE ESTUARIES OF SOUTH WEST COAST OF INDIA" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or other similar titles.

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सारांश

द्विकपाटियों को सामान्यतः निस्यंदक भोजी माना जाता है क्योंकि प्रदूषकों को कई मात्राओं में सकेंद्रित करने में उनकी बडी क्षमता होती है. वर्तमान अध्ययन के लिए पृथुलवणी तथा हार्डी स्वभाव वाली काली सीपी *विल्लोरिटा साइप्रिनोइड्स* को चुना गया. चार स्टेशनों में (स्टेशन 1,2,3 और 5) किए गए पाक्षिक अध्ययन और अन्य चार स्टेशनों में (स्टेशन 4,6,7 और 8) किए गए मौसमिक अध्ययन से व्यक्त हो गया कि सीपियों की पाचक ग्रंथि के पूरे मृदु ऊतक में मानसून के बाद कोपर की अधिकता और मानसून के पूर्व न्यूनता है. कैड्मियम और लेड की मात्रा में तुलनात्मक कमी दिखाई पडी. फिर भी तलछट में मौजूद लोहों के जमाव में कोई मौसमिक परिवर्तन नहीं देखा गया. तीव्र आविषालुता और जैव संचयन अध्ययनों (कोप्पर और लेड उपयुक्त करके) द्वारा यह दिखाया पडा कि सीपियों केलिए लेड की अपेक्षा कोपर अधिक विषैला है. पाचक ग्रंथियों में कोपर की अधिकता और क्लोम में लेड की अधिकता थी. अध्ययन किए गए ऊतक में मौजूद दो लोहों के संचयन में समय और सांद्रता का रेखीय संबंध दिखाया पडा. सीपियों में कोपर तथा लेड युक्त पाचक ग्रंथि तथा क्लोम का ऊतक विश्लेषण करने पर दिखाया पडा कि इन लोहों द्वारा उपकला कोशों का विपोषण या विनाश हो जाता है. इस पर आयोजित प्लैनीमीटरीय अध्ययन द्वारा भी यह व्यक्त हो गया कि कोपर युक्त सीपियों में दिखाए जाने वाला कम एम ई टी मूल्य (Mean Epithelial Thickness) उपकला कोशों के विनाश का सूचक है. कोपर युक्त सीपियों में एम एल आर तथा एम एल आर/एम ई टी की मात्राओं में वृद्धि दिखाई पडी. लेड युक्त सीपियों में समान परिणाम देखे जाने पर भी सांख्यिकीय विभिन्नता नहीं दिखाई पडी. कोपर तथा लेड युक्त सीपियों में देखी गई अच्छी संरचना की पाचक ग्रंथि से व्यक्त हो गया कि इन सीपियों में एस ई आर, लाइसोम्स का प्रचुरण, पाचक कोशों का नाश, परिवर्ती केंद्रक कला और आधार पटल में विच्छेद हो जाता है.

ABSTRACT

Bivalves which are filter feeders are found to be the best ones suited for pollution monitoring because of their abiltiy to concentrate pollutants to several orders of magnitude. For the present study, black clam, *Villorita cyprinoides* was selected owing to its euryhaline and hardy nature. A fortnightly study conducted at the four stations (station 1, 2, 3 & 5) and a seasonal (Pre-monsoon, Monsoon & Post-monsoon) study conducted at the other four stations (station 4, 6, 7 & 8) revealed a post-monsoon maxima and a pre-monsoon minima for copper in the whole soft tissue and digestive gland of clams. Cadmium and lead values were comparatively low. However, metals in the sediment did not show any seasonal variation in accumulation. Acute toxicity and bioaccumulation studies (using copper and lead) showed that copper was more toxic to the clams than lead. Digestive glands showed highest value for copper whereas gills showed highest value for lead. A linear relationship between time and concentration was observed in the accumulation of the two metals in the tissues studied. Histology of the digestive glands and gills of copper and lead exposed clams showed severe degeneration and destruction of the epithelial cells. The planimetric study conducted also revealed destruction of epithelial cells which was indicated by a significantly lower MET(Mean Epithelial Thickness) values for copper exposed clams. There was Significant increase in MLR & MLR/MET values of copper exposed clams. Though same results were obtained for lead exposed clams the values did not show any statistical difference. Fine structure of the digestive glands of copper and lead exposed clams revealed proliferation of SER, lysomes, destruction of digestive cells, a labile nuclear membrane and breakage at the basal lamina.

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1. INTRODUCTION

The tremendous increase in the growth of human population and the subsequent development of industries have resulted in the indiscriminate use of many harmful chemicals including heavy metals. Their continuous release into the environment has transformed the coastal zone into an area which is highly vulnerable to the harmful effects of these pollutants. Periodically public attention is drawn to what is arguably the most insidious and dangerous category of pollutants in the sea; heavy metals. Once added to the environment they are not readily degradable and they are often accumulated in the tissues of organisms. Since there is no mechanism of selective uptake or excretion, in certain cases this leads to an amplification of the concentration of metals in animals higher up the food chain. Man, as a carnivore, therefore, is exposed to the risk of taking a diet enriched by these substances and several metals are cumulative poisons, causing among other things, brain damage at low dose rates and death at very high rate.

Metals are immutable, they can neither be created nor destroyed nor can one metal be transformed into another. Of the heavy metals, some like copper and zinc have some biological role in the living systems, whereas metals like lead, cadmium, mercury and arsenic have no such known function. Their continuous release into the environment can alter the normal metal cycles posing serious threat to the life of plants and animals including man (Holden, 1973; Vernberg & Vernberg, 1974). Once

they enter the food chain, heavy metals cannot be removed from the system and will certainly pass on to higher trophic levels. Hence, metal pollution is of great concern in the management of marine and estuarine ecosystems (Quasim & Siddiqui, 1960; Azariah, 1985).

In the long term, persistant discharges of pollutants, however inconspicuous it may be, affect the economy (fishes, other sea foods, coastal waters & estuaries), human health and biodiversity. Taking the above factors into consideration, this category of contamination needs special care. Environmental research has suggested that some bivalves may be valuable as sentinel organisms for indicating levels of pollutants in coastal marine waters. These organisms concentrate pollutants to a marked degree over sea water levels. A given species, however, may have unique enrichment factors for any or several groups of pollutants. In this context, in the last decade, biomonitoring programmes have been developed in order to assess the quality of an environment withstanding persistant incoming of pollutants especially of trace metals in coastal and estuarine waters (Phillips, 1977; 1978; 1980; Bryan et al., 1980; Phillips & Rainbow, 1993; Goldberg et al., 1978). The use of biomonitors to establish geographical and/or temporal variations in the bioavailable concentrations of trace elements in coastal and estuarine waters is now well established (Phillips, 1980; Bryan, 1976; Rainbow et al., 1990). Biomonitors provide time- integrated measures of the levels of available metals in their ambient waters, responding essentially only to the ecosystem that is of direct ecotoxicological relevance. Biomonitors as the name implies are organisms which help us in assessing the quality of an environment. These organisms can

accumulate pollutants including metals in their soft tissues to concentration of several orders of magnitude above ambient levels (Bryan, 1976; Rainbow et al., 1990). Measurement of metals concentrated by these animals is more easier and less laborious when compared to the analysis of sediments and sea water.

The interpretation of metal results from sentinel organisms can be complexed by a variety of factors. For example, concentrations can be a function of animal size, age, season of sampling, sex of the organism, its vertical position on the shore line, the salinity and temperature of the ambient environment. Many of these parameters may be interrelated.

In the present study, black clam (*Villorita cyprinoides*) is used as the sentinel organism since it is one of the important species available in the lakes and estuaries in the region and also used for human consumption as well as fish feed. About 37036.2 tonnes (Annual Report, CMFRI, 1996-97) are fished annually. This organism being a bivalve and a filter feeder can accumulate toxins in its soft tissues which forms the edible portion of the animal. Its meat being cheap and nutritious forms the food for the common man whereas the shell is used in making cement, lime and also used in poultry. The clam meat is also used for feeding shrimps particularly breeders in culture systems for ensuring early maturity and good spawning rate owing to the presence of certain essential fatty acids. It is estimated that the meat portion is about 3700 tonnes (10%), of which approximately 40% (1400 tonnes) is utilised for human consumption and the remaining 60% (2300 tonnes) is used for other

purposes like poultry, fish feed etc. A large number of labourers subsist on this resource either directely or indirectely having a significant role in the socio-economics of the region.

The environment selected

The backwaters extending from Alleppey to Cochin, known as Vembanad lake is the largest brackish water system in Kerala having a net work of canals and rivulets, owing to the high productivity and extremely favourable physical and biological conditions for growth and propagation, the animals associated with these brackish water environment are rich and varied.

The pollution caused by the discharge of partially treated or untreated wastes from the factories, sewage and chemicals from agricultural operations (fertilizers as well as pesticides) finding their way into the water bodies are the main factors contributing to the water pollution problems in this estuarine ecosystem.

Of late, there have been several reports of fish kills as well as significant reduction in the catch of some of the important fishery resource from this lake system. One of the reasons attributed to the same is degradation of water quality due to various pollutants including industrial and agricultural wastes (Pillai, 1991).

The estuarine habitat selected for the study fulfills certain

conditions as proposed by Clark & Cripe(1993) which makes it best site for regular monitoring.

- These habitats are accessible

- Sources of pollution are located within estuaries, they can be regarded as a sink where large quantities of water and waste come from drainage

- River run off and most part of it will settle down and some are washed off shore during hightides

- Moreover they are the areas having considerable economic importance from a fishery point of view.

It is expected that the present study conducted may pave way for future research on designing the strategies and management plans that should be followed, so as to check the level of heavy metal pollution in the area as well as the factors affecting the distribution and abundance of clam population.

Objectives of the study are:

1. To study the level of metal(copper,cadmium and lead) pollution in the cochin backwaters.

2. To study the toxicity of metals (copper and lead) to *V.cyprinoides.*

3. To study the histological and ultrastructural changes occurring in *V.cyprinoides* due to metal toxicity.

4. To assess the suitability of choosing *V.cyprinoides* as a biomonitor for future research.

2. REVIEW OF LITERATURE

The ecological significance of heavy metals as stressed by some earlier workers (Keith & Telhard, 1979; Purves, 1985) could be due to their toxicity and cumulative behaviour with serious public health implications (Phillips & Rainbow,1993). Unlike other pollutants they are not bio-degradable hence undergo a global ecobiological cycle (Nürnberg,1984) in which natural waters are the main pathways. Many hazardous substances discharged into the aquatic environment are known to accumulate in river or estuarine sediments (Forstner & Muller, 1973).

2.1 Heavy metals in sediments

Normally sediments are not subjected to much variations when compared to the overlying water body. Sediments act as traps for various compounds in the aquatic system. Hence the analysis of sediments is a good indicator of water quality (Förstner & Salmons, 1980).

Copper is regarded as a wide spread pollutant in industrialised estuarine areas and is relatively more toxic than other essential trace metals(Sosnowski& Gentile, 1978; Johnson & Gentile, 1979; Sullivan et al., 1983; Sunda et al., 1987; Paulson et al., 1989; Ahsanullah

& Williams,1991).

For Copper, varying values in different seasons were obtained in a study conducted by Venugopal et al.(1982). There are a number of reports regarding the fate of metals in estuarine waters (Gibbs, 1973; de Groot, 1973; Dunicker & Nolting, 1976). Flocculation and sedimentation of colloidal and fine material in suspension may cause a net setling of trace elements (Dyer, 1972). A net sedimentation of metals in estuaries was also reported by Turekian (1971).

The Cochin estuarine system enlisted as a polluted estuary, receives contaminated fresh water inputs and discharges of crude sewage, trade effluents and partially treated sewage (Jayasree & Nair, 1995; Remani et al., 1981; Paul & Pillai, 1983; Balchand & Nambissan, 1986).

Aquatic animals are exposed to both essential and non essential metals present in the aquatic environment. Essential ones support biological processes whereas non essential ones does not have any role. Trace metals taken up by marine invertebrates are accumulated to high body contents and concentrations. Cellular functions are critical to processes involved in metal uptake, regulation, utilization and release. Toxicity can be due to their dysfunction and the resultant interaction of metals with inappropriate cellular structures. The uptake of metals affected by many factors including extrincic physico chemical factors controlling the metal bioavailability like dissolved metal concentration, temperature, salinity, presence or absence of other metals and intra and inter specificity, varying intrincic factors such as surface impermeability, nutritional state,

stage of moult cycle and filtration rate (Phillips,1980).

2.2 Heavy metals in different organisms

In order to express their toxicity, which inhibits growth in plants, animals and micro organisms, heavy metals generally have to enter living cells (Arnebrant et al., 1987; Rauser, 1990; Steffens, 1990; Ernst et al., 1992; Schat & Kalff, 1992). Heavy metals have been reported in some aquatic fungi (Duddridge & Wainwright, 1980; Abel & Barlocher, 1984), in the yeast *Saccharomyces cerevisiae* and a few other species (Winkelmann & Winge, 1994). Many organisms have evolved tolerance towards heavy metals (Verkleij & Schat, 1990; Robinson et al., 1993). As a consequence they can accumulate to high concentrations of these metals without suffering any deleterious effects and this forms the core issue of many studies reported from Indian waters (Sankaranarayanan et al., 1978; Agadi et al., 1978; Kureishy et al., 1981; Lekshmanan & Nambisan, 1983; Patel et al., 1988; Pillai et al., 1986).

Jaffer & Ashraf (1988) reported heavy metals like copper, cadmium,lead,arsenic etc from the muscle,liver and kidney of long tail tuna*(Thunnus tonggol)* and Indian oil sardine from the coastal waters of Pakistan. High levels of Cu & Zn have been noticed in *Metapenaeus brevicornis* and *Penaeus hardwicki* and in crab *Scylla serrata* from Thana creek. Comparatively higher levels of copper and zinc reported from *Charybdis* sp. from Bassein Creek (Asha Jyothi & Vijayalakshmi, 1999).

Some of the earlier works (Bryan, 1973 ; Pentreath, 1973; Majori et al., 1978; Phillips, 1980) have shown seasonal patterns of tissue metal accumulations in bivalves.

Indigenous molluscs are so often used as indicators in pollution studies because of their sensitivity to copper, zinc and other associated metals, (Wurtz, 1962; Rehwoldt et al, 1973; Cairns et al., 1976; Couhtrey & Martin, 1977; Guth et al., 1977; Cherry et al., 1980), sedentary and comparatively long life, position in the food web and accessibility (Cairns et al., 1971). The gills, digestive tract and integument represent the sites of metal uptake. Metals are subsequently transported to internal organs for utilization, storage and release. Components of hemolymph are the vehicles of their transport. Usually, in oysters metals bind to haemocytes (George et al., 1978). Cellular mechanisms associated with various organ systems operate in a highly integrated manner to co-ordinate metal uptake, transport and release. Thomson, (1979) reported heavy metals (cadmium & lead) in the native oyster *(Oystrea augasi)* and mussel *(Mytilus edulis)* from Port Davey, South Western Tasmania.

Zingde et al. (1976) reported some heavy metals in *Crassostrea cucullata* and *Crassostrea gryphoides* from Goa. Heavy metals in the tissues of wild *Mytilus edulis* have been reported at a number of localities around the Australian coast line (Harris et al, 1979; Wootten & Lye, 1982). Copper, lead and zinc were also reported in mussels (*M. edulis*) from Halifex inlet (Rickardo E. Ward, 1990).

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Clams have been shown to exhibit the capability of concentrating a wide variety of pollutants within their tissues and hence they were regarded as good indicators of environmental pollution. Pillai & Valsala(1995) reported seasonal variations of copper, cadmium and lead in the bivalve *Sunetta scripta* from Cochin coastal waters. Seasonal variation of copper, zinc and manganeese in cultured mussel *Perna viridis* and sea water in Don Paula Bay (Goa) was reported by Rivonker & Parulekar (1998). In this study they found that young mussels recorded high values and the accumulation of copper was influenced by salinity and proximity to point source. In a study of heavy metal exchange among the aquatic environment in the Mediterranean coast, FE EI-Nady (1996) reported that immature animals accumulate metals like copper, zinc, iron and lead to a high rate compared with the mature ones and also found that increased concentrations of these metals caused a reduction in growth rate and reduced the animal's condition index. Rajendran et al, (1988) reported measurable levels of copper from *Crassostrea madrasensis* in Cuddalore backwaters(South East coast of India) and reported high level of accumulation in gills and mantle.

Studies comparing the relationship between the major differences in geochemical characteristics of the sediments and water and the speciation and bioavailability of the pollutant had been carried out by several workers (Bryan 1980; 1984; Senthilnathan et al., 1998; Harrison, 1985; McLusky et al, 1986; wang, 1987; Flemming & Trevors, 1989; Millward, 1995).

The differences in the physiological state of the organisms, influencing the accumulation and elimination kinetics in the organisms has been studied by Bryan, (1980); (1984); Senthilnathan et al., 1998; Wang, (1987); Viarengo, (1989); Livingstone & Pipe, (1992). Strong & Luoma, (1981); Cain & Luoma, (1986); (1990); Soto et al., (1995); Classy D' Slva & Kureishy, (1978). Soto et al., (1995b) have reported body size of an organism as an important factor influencing metal accumulation rate in it. Similar studies relating size and rate of metal accumulation in mussels have been carried out by Simpson, (1979); Cossa et al., (1980); Orren et al., (1980); La Touche & Mix, (1982); Ritz et al., (1982); Popham & d' Auria, (1983).

Heavy metal, mainly copper has been studied in black clam, *Macoma balthica* (Strong & Luoma, 1981; Cain & Luoma, 1986; 1990; Bryan & Langston, 1992) because of its high potential toxicity and its abundance in some estuaries. A seasonal distribution of metals (Cu^{2+}, Cd^{2+}, Pb^{2+} & Zn^{2+}) with high levels during monsoon season was observed in different organs like gill, mantle and adductor muscle of the oyster *Crassostrea madrasensis* has been reported from the Uppanar, Vellar and Kaduviar estuaries of southwest coast of India (Senthilnathan & Balasubramanian, 1998). Seasonal variation of metals (Cu^{2+}, Zn^{2+}, Pb^{2+} & Hg^{2+}) indicating a monsoon maxima and a summer minima has been reported in mussel *Perna viridis* and oyster *C. madrasensis* from some parts of south east coast of India (Senthilnathan et al., 1998; UNEP, 1982; Sunderaraj & Krishnamurthy, 1972; Lekshmanan & Nambisan, 1983).

The relationship between concentration of heavy metals (Pb^{2+}, Cd^{2+}, $C2^{+}$, Zn^{2+}, CO^{2+}, Ni^{2+} & Ag^{2+}) in bivalve molluscs (*Area ventricosa, Chama isotoma, Lithophagateres, Pinctada margaritifera, Pycnodonte hyotis,Spondylus ducalis, Modiolus auriculatus, Trichomya hirsuta,Ustularea renute*) and their environment were studied by Klump & Burdon-Jones(1982). A correlation has been noticed between metal (Pb^{2+} & Cd^{2+}) levels and abiotic factors (salinity & source) in a study conducted in *Mytilus edulis* from the estuary and Gulf of St. Lawrence, Canada (Cossa & Bourget, 1980). According to some workers (Phillips, 1976; Wright, 1977) low salinities favours an increase in the uptake of heavy metals.

Lamellibranch molluscs are known to concentrate heavy metals to levels far in excess of those in the hydrosphere (Brooks & Rumsby, 1965; Bryan, 1973; Romeril, 1974; Schulz Baldes, 1974; Watling & Watling,1976).

Claude & Maureeni(1999) conducted a study signifying the so called "dilution effect" where difference in metal levels in the samples (*Anadara senilis*) has been attributed to differences in size groups. Several workers (De Wolf, 1975; Boyden, 1977; Bryan & Hummerstone, 1978; Davies & Pirie, 1980) suggested an increase in metal concentrations with age. Roberts et al., (1986) has found that younger mussels had a higher uptake of Cd^{2+} because of their higher metabolic activity and feeding period. Williamson(1980) observed Cd^{2+}, Pb^{2+} & Zn^{2+} in a population of snails where he attributed differences in metal accumulated to ages or sizes. Jackim et al., (1977) and Mac Innes & Calabrese, (1979) had observed

that low salinity increases toxicity of cadmium and copper.

A correlation between salinity and trace metal contamination has been established in *C. virginica* (Phelps et al., 1985). An inverse relationship between cadmium accumulation and salinity was found out by Gold-Bouchot et al. (1995).

Rijstenbil et al. (1998) has found the influence of nitrogen status on copper accumulation in planktonic diatom, *Thalassiosira pseudonama*. Joseph & Srivastava(1992) studied the heavy metals (Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+} & Cr^{2+}) in *Penaeus indicus* from Ennore estuary.

Effects of heavy metals (Zn^{2+}, Cu^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} & Pb^{2+}) had been studied in edible *C. cucullata* from Hooghly estuary (Abhijit mitra et al., 1996).

Wilfred and Aziz(1994) has reviewed the potential of estuarine bivalve *Paphia malabarica* as a bioindicator of copper pollution. Ramalingam Pillai et al.(1994) studied metal level in sediment and water along south west coast of India from Cochin to Veli. They also studied inorganic phospate, nitrite-nitrogen, nitrate-nitrogen and ammonia nitrogen values in these regions.

Ananda Gupta et al. (1996) in a study had established a relation between Zinc accumulation in oyster *C. cucullata* and biological (breeding season) as well as some ecological factors (salinity& pH). Effects of season and

size on heavy metal concentration in the common cockle *(Cerastoderma edule(L.)* was elucidated by Savari et al., (1991). Ouseph (1987) has reported seasonal variations in heavy metal concentrations in Cochin estuarine system.

Amiard et al. (1986) conducted an ecotoxicological study of Cd^{2+}, Pb^{2+}, Cu^{2+} & Zn^{2+} in the mussel *Mytilus edulis*. They have shown that seasonal variations in Cd^{2+}, Cu^{2+} & Zn^{2+} concentration in *M.edulis* are characterised by summer minima and winter and spring maxima.

Metal (Cu^{2+}, Cd^{2+} & Zn^{2+}) accumulation in the clam *Tridacna crocea* under natural and experimental conditions were studied by Duquesne & Coll(1995) and found high concentration of Copper in the gills and Cadmium in kidney.

Heavy metals in the clam *Mercenaria mercinaria* was studied by Robert & Trefry(1996). In this study, no significant correlation between metal concentrations in the clams and those in either sediment or water were found. Similar observation was made by Presley et al. (1990) for oysters in Gulf of Mexico.

Boyden (1977) found that concentrations of Zinc in *M. edulis* was greater in the smallest individuals when compared to the larger size groups. Boyden & Phillips (1981) reported that metal concentrations in oyster *C.gigas* were related to tissue weight of the oyster which in turn was related to gametogenesis and spawning.

Heavy metal pollution studies in oysters *C.virginica* has been conducted by several workers (Hugget et al., 1973; Frazier, 1975; 1976; 1979; Burrell et al.,1981;Zamuda & Sunda, 1982). Especially high levels of Copper and zinc have been reported in oysters (Goldberg et al., 1978; Martincic et al., 1984).

2.3. Toxicity Studies

For the evaluation of the effects of a particular metal on animals, toxicity tests are conducted. The most frequently recorded result of a toxicity experiment is the death of the organism. In the exposure studies, animals are exposed to a given concentration of a metal and results are defined in terms of that concentration (GESAMP, 1972). There is considerable value in obtaining the concentration/response curve for the metal under the specific test conditions, which requires that observations of the test apparatus should be made at frequent intervals to record the individual survival times. So that for each concentration the median survival time can be calculated.

Somewhat less information can be obtained from an inspection for mortalities at infrequent fixed intervals from which LC50 values for these times can be derived, i.e. at 6, 12, 24, 48, 96 hrs. Ideally the test should be continued until a threshold concentration (where the concentration/response curve become parallel to the time axis) is established. The slope of the toxicity curve can give some indication of the reactions

of the test organism to the metal.

Studies on acute and chronic toxicity of copper and mercury in green mussel *Perna viridis* was done by Sree Kala Pillai & Menon (1998). Mathew and Menon (1992) studied toxic responses of *Donax incarnatus,* exposed to copper, cadmium and mercury and their combinations and found that the tropical coastal waters with high dissolved organic matter influence the toxicity of metals. Active accumulation of cadmium in the digestive gland of mussels was reported in a thirty day exposure study (Da-Ros et al, 1993).

Effect of cadmium exposure in crab *Scylla* (Forskall) has been elucidated by Dhavale & Masurekar(1986).Wildgust & Jones (1998) has investigated the mysid *Neomysis integer* at different salinities in relation to free cadmium ion concentrations.

It is well established for aquatic animals that the toxicity of most trace elements increases with decreasing salinity (Jones, 1975; Mc Lusky et al., 1986; De Lisle & Roberts, 1994; Hall & Anderson, 1995). For any metal to be toxic it must be accumulated hence, metal toxicity is dependent upon metal bioavailability. Several workers (Schulz-Baldes, 1974; Majori & Petronio, 1973; Scotth & Major, 1972; George et al., 1978;) had conducted short duration exposure studies in mussels.

Bebiano et al, (1993) studied cadmium uptake, storage and

metabolism in the clam *Ruditapes decussata.*

In Rainbow trout *Onchorhychus mykiss*, effect of acute exposure to diatery cadmium and copper were studied (Handy, 1993). Toxicity study conducted in green mussel *P.viridis* on the accumulation of copper and zinc (Classy D'silva & Kureishy, 1978) revealed the fact that the metal uptake by mussels is directly proportional to the external concentration. Pringle et al, (1968) also obtained the same result.

Acute toxicity of copper to a copepod has been studied by Moraitou-Apostolopoulou (1978). Studies were conducted by different authors to evaluate the influence of environmental factors on copper toxicity (Mc Kee & Wolf, 1963; Doudouroff & Katz, 1953; Bender et al., 1970)

Studies illustrating the effect of combination of metals in mussels have been carried out by several workers (Davies & Russel, 1988) in clams (Patel et al., 1988) and in sea stars (Sorrensen & Bjerregaard, 1991) in crabs (Bjerregaard, 1982; 1985; 1988).

Krishnakumar et al., (1987a) has studied acute mercury and zinc toxicity in green mussel *P.viridis*. Doudoroff and Katz (1953) studied the toxicities caused by lead, copper, zinc and mercury in mussels.

Chan (1988) had studied the accumulation and tolerance to cadmium, copper, lead and zinc by the green mussel *P.viridis*.

Among heavy metals, copper is found to be the most toxic one (Viarengo, 1989; Davenport & Redpath,1984). Uptake and accumulation of cadmium by *M.edulis* was reported by George and Coombs (1977). Lead is found to be a bio accumulative toxic metal and has no role in physiology of the organisms (Wood, 1974; Waldichuk, 1980).

Phillips (1978) studied the variables that affect lead kinetics in the mussel *M.edulis*. According to him high concentration of metal was encountered in low saline conditions. According to Tan & Lim (1984) bivalves has the ability to concentrate lead several times higher than that present in the medium.

Denton & Jones (1981) studied the influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the black lip oyster *Saccostrea echinata.*

Effect of temperature on cadmium uptake was studied in a number of animals like Fiddler crab *Uca pugilaltor* (O' Hara, 1973), the blue crab Callinectes sapides (Hutcheson, 1974) and the marine mussels *M. edulis* (Phillips, 1976a). Kluytmans et al.,(1998) studied the effects of cadmium on the reproduction of *M.edulis.*

Copper is found to be the most toxic metal for bivalves (Krishnakumar et al., 1987b; Scott & Major, 1972; Grace & Gainey, 1987; Viarengo, 1989) and its rate of accumulation was found to be greater than mercury (Krishnakumar, 1987b; Krishnakumar et al., 1990a).

High level of cadmium accumulation was reported in the hepatopancreas of *M. edulis* (Theede et al., 1979).

2.4. Histology

Histological diagnosis at the light microscope level is of great help in monitoring the effects of pollutants like metals (Papathanassiou & King, 1986; Marigomez et al, 1990a) in a variety of invertebrates. Histology as a tool for examining cellular changes (morphological and structural) have been used in several studies (Cajaraville et al., 1989a; Marigomez et al., 1990a; 1990b). Vega et al., (1989) studied the effects of sublethal concentrations of cadmium on the digestive cell morphology of *Littorina littorea*. Handy (1992a), Harrison & Klaverkamp (1989) had stated that about 75% of metal burden is supported by gut. The digestive gland of molluscs shows a great natural plasticity and the morphology of the digestive tubules changes considerably with alterations of environmental conditions (Thomson et al., 1974; Lowe et al., 1981; Moore, 1988b; Couch, 1984; Axiak et al., 1988; Vega et al., 1989; Marigomez et al., 1986; Recio et al., 1988).

The digestive gland metal content in mussels have been proposed as a reliable indicator of metal pollution for the case of non-essential metals (Regoli & Orlando, 1993; 1994 a;b). Similar observations were made in the case of marine winkles (Soto et al., 1997). Bright (1987) studied the histopathological changes occurring in gastro

intestinal epithelia, kidney and gills of *Macoma carlothensia* due to copper toxicity.

Morphology of digestive gland in bivalves has been studied in detail by several authors (Owen, 1972; Merdsoy & Farley ,1973; Langston, 1975; morton, 1983; Robinson, 1983). Changes in the tubule structure has been observed in several studies (Thomson et al., 1974; Pauley & Sparks, 1965; Moore et al., 1978 a;b; Lowe et al., 1981; Couch, 1984; Tripp et al., 1984; Marigomez et al., 1986; Axiak et al., 1988; Recio et al., 1988; Marigomez et al., 1985). Atrophy of the digestive cells, dialation and degeneration of the digestive tubules etc were reported in some of the earlier works (Lowe et al., 1981; Calabrese et al., 1984; Lowe, 1988). Lowe & Clark (1989) reported alterations in the lysosomal vacoular system and a reduction in the volume density of the digestive epithelium combined with an increase in its specific surface was reported in the digestive cells of *M.edulis* exposed to a mixture of copper and hydrocarbons.

Some workers have shown that bivalves generally concentrate heavy metals to a greater degree in the gills and visceral mass compared with the mantle and adductor muscle (Segar et al., 1971; Bryan, 1973; and in laboratory investigations by Brooks & Rumsby, (1967); Pringle et al., (1968); Preston, (1971); Romeril, (1971); Pentreath, (1973); Kopfler, (1974); Cunningham & Tripp, (1975); Denton & Burdon-Jones, (1981), showed that the digestive gland of *M.edulis* accumulated more cadmium than other soft tissues. In a study on accumulation and toxicity of copper in *P.orientalis*,

Liu Fayi et al., (1988) reported maximum accumulation of copper in the hepatopancreas.

Martin (1971) showed histopathological changes in the digestive tubules, gills and mantle epithelium of the Asiatic fresh water clam *Corbicula fluminer* exposed to copper. Fujiya (1960) also observed histopathological changes in the digestive diverticula and stomach of oysters. Krishnakumar et al., (1990a) in an exposure study conducted on *P.viridis* found that more copper was accumulated in the digestive gland than in other tissues.

Exteberria et al., (1994), has carried out an investigation to quantify changes like structure of the digestive lysosomes of mussels *M. galloprovincialis*, which was exposed to sublethal concentrations of metals in both field and laboratory condition. Tubule dialation, breakdown and cilia loss in the digestive diverticula of digestive gland were reported by Krishnakumar et al., (1990); Calabrese et al., (1984); Lowe, (1988); Aufret, (1988).

Epithelial disruption and necrosis were observed in *M.edulis* by Rasmussen (1982). Similar changes were also noticed in the scallop *Placopecten magellanicus*, following cadmium and copper exposure (Yevitch & Yevitch, 1985). Degenerative changes ranging from inflammatory response to extreme vacuolation was noticed in the digestive gland of marine mussels and clams following exposure to copper and cadmium (Sarasquete et al., 1992).

Digestive gland was shown to play a key role in metal accumulation in winkles(*L.littorea*), (Mason & Simkiss, 1983; Marigomez et al., 1990b ; Nott et al., 1993).

2.5. Planimetry

Quantitative methods for assessing morphology of digestive tubules have been developed using direct measurements (Marigomez, et al., 1985), stereology and planimetric techniques (Recio et al., 1988). The planimetric procedure has been used by several workers for a number of bivalves like *C. virginica* (Couch, 1984) and *M.edulis* (Langton, 1975).

Planimetric study as a tool for measurement of structural changes in the digestive tubules of bivalves and gastropod has been proposed by several authors (Marigomez et al., (1986b); Recio et al., (1988); Cajaraville et al., 1992b; Marigomez et al., 1990; Marigomez & Ireland, 1990). Vega et al., (1989) studied the qualitative alterations in the structure of the digestive cell of *L.littorea* exposed to cadmium using the planimetric procedure. Marigomez et al., (1992) studied the seasonal variability in the quantitative structure of the digestive tubule of the gastropod using the same procedure. Soto et al., (1990) conducted a planimetric study to know the morphological variability in the digestive diverticula of the gastropod *L.littorea* and the mussel *M.edulis*. The planimetric procedure was also used (Cajaravilla et al., 1989a; 1992b) to assess the degree of stress experienced by mussels when they were kept under laboratory conditions.

Gills are found to be the main site of metal uptake in molluscs (George & Pirie, 1980; Roesijadi & Unger, 1993; Brough & White, 1990). Gills support upto 40% of the body burden when water borne exposure to either Cadmium or Copper occurs (Handy, 1992b; Harrison & Klaverkemp, 1989).

In the whelk *Busycon canaliculatum* copper caused necrosis and sloughing of the gill epithelium (Betzer & Yevich, 1975). Similar observations were made in hard clam, *M.mercenaria* exposed to copper for 14 weeks (Yevich, unpublished). Sloughing of ciliated gill epithelial cells was noticed following copper exposure in mussels maintained at high salinity (Miller, 1989).

2.6. Ultrastructure

The knowledge at the cellular and sub-cellular levels of organisation is of great help in understanding the impact of pollutants to the higher levels of biological organisation (Cajaraville et al., 1993) thus providing rapid and highly sensitive indicators of environmental impact (Moore, 1985). Lysosomal system in the digestive cells of bivalves was a subject of several important studies because of the role played by these organelles in the intracellular digestion. Various structural changes associated with lysosomes due to contaminant exposure were reported earlier (Marigomez et al., 1989; Cajaraville et al., 1995). Lysosomes are important structures in metabolism and storage of metals (Viarengo, 1989;

Marigomez et al., 1990b). Reduction in lysosomal membrane stability have been recorded after experimental exposure of mussels to copper (Viarengo et al., 1981; Harrison & Berger, 1982), cadmium (Viarengo et al., 1987), methyl mercury and selenium (Pellerin-Masicotte et al., 1989) and anthracene and phenanthrene (Nott et al., 1985).

Marigomez et al., (1990) reported extensive proliferation of smooth endoplasmic reticulam in the excretory cells of the renal epithelium of cadmium treated *Littorina littorea.* Similar observation was reported in *Mytilus edulis* exposed to polynuclear aromatic hydrocarbons (Nott et al., 1985). Morphological changes of mitochondria as a result of stress was reported earlier (Marigomez et al., 1990a; Smith & Ord, 1983).

3. MATERIALS AND METHODS

3.1. Sample collection for field study

3.1.1. Collection of water sample

Water samples from the selected sites were collected in clean, acid washed plastic bottles and were immediately brought to the laboratory for analysing the hydrological parameters (pH, salinity, po_4, No_2 & No_3). Temperature of the water was taken at the collection spot itself using a Mercury thermometer. For the analysis of hydrological parameters, the procedure suggested by strickland & Parson (1968) was followed.

3.1.2. Collection of sediment

Bottom sediment from the sites were collected using a clean plastic spatula and was stored in clean polythene bags and were brought to the laboratory for estimation of heavy metals (Copper, Cadmium, Lead).

3.1.3. Collection and storage of biological samples

Live clams, *V.cyprinoides* of uniform size (30-40mm) were

collected in fresh polythene bags and were placed inside an icebox and were immediately brought to the laboratory and stored in deep freeze. Two size groups (20-30mm) and (30-40mm) were selected from the two stations (Panangad and Chembu) to know the size related variations in metal accumulation based on the concept of selecting animals in the age group below one year and above one year. A homogenous population (30-40mm) was selected from the other two stations (Station 1 & Station 5) for regular sampling (fortnightly) and four other stations Thannirmukkom, Muhamma, Vaduthala and Pallipuram were selected for a seasonal study.

3.1.4. Collection and transportation of live clams for bio-assay

Healthy clams of uniform size from an unpolluted site were collected in polythene bags filled with clean brackish water of 10ppt salinity and the temperature of the water noted and were immediately brought to the laboratory with minimum stress.

The fortnightly collection centres like Panangad, Chambakara, Konthuruthy and Chembu will be refered as stations 1, 2, 3 & 5 and centres like Vaduthala, Pallipuram, Thannirmukkom and Muhamma, where samples were collected on a seasonal basis (Pre-monsoon, Monsoon & post-monsoon) will be refered as stations 4, 6, 7 & 8 respectively hereafter (Fig:I).

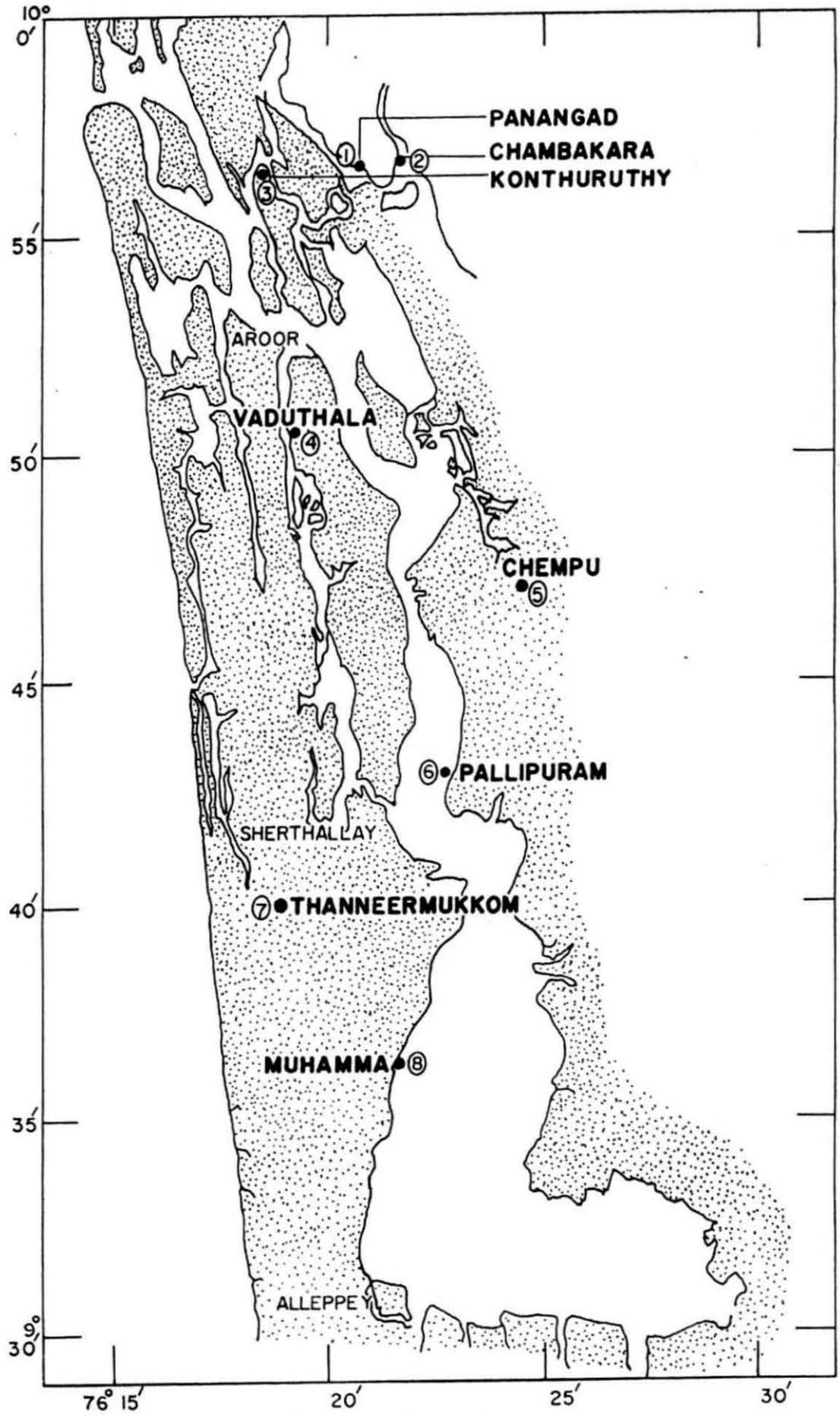

Fig I : Stations selected for the present study

3.2. Acclimatization

In the laboratory, animals were kept in polythene tubs of 10 l capacity. The tubs were soaked in acid for 24 hrs and washed with copious amounts of deionized water. Fine meshed nets were provided for the tubs to protect from dust during the experiment. The animals were acclimated for two days in the tubs with clean brackish water of 10 ppt salinity and were fed with algal culture. Aeration was also provided.

3.3 Test solution

1000 ppm solution of Copper was prepared by dissolving AR grade $CUSo_4.5H_2O$ in double distilled water. For Lead, standard solution of lead was used.

Calculated quantities of the solutions were added to the experiment tubs containing a known volume of brackish water to obtain the required test concentrations.

3.4. Acute Toxicity Studies

The procedure given by APHA et al., (1981) was followed in the experiment. For the study, filtered, unpolluted brackishwater of 10±2 ppt salinity was used. Other parameters like pH 7.8±.1 and dissolved oxygen

>90 % saturation were kept constant. Test animals of uniform size of 28-35 mm were sorted and cleaned for the experiment. After conducting a pilot study, eight concentrations were used for each metal.

Each tub contained 5litre brackish water and 10 clams. One tub was kept as control without metal solution and duplicates were run for each metal concentration. Clams were inspected every 12 h for mortality and water was renewed every 24 h. Clams were considered dead if they gaped the shell wide and showed no response to tactile stimulus. Dead clams were removed and recorded every 12 h.

3.5. Data analysis

3.5.1. Sub-lethal studies

3.5.2. Experimental procedure

Clams were collected, cleaned and acclimated for 5 days in the laboratory as explained in section 3.1.3. Clams were fed with algal culture.

Clams of uniform size (28-35 mm) were exposed to sublethal concentrations of Copper and Lead. Stock solutions of metals were prepared and added to the medium as described in section 3.3. For each

metal, two concentrations were used. The concentrations of the two metals used and the physico-chemical parameters of the experimental system were given in the table 3(i). The test medium was changed everyday and 8-10 clams were removed from each tub after every 7 days from the start of the experiment for e.g. 0 hr, 7 days, 14 & 21 days to determine the background concentration of metals in the tissues. Control tubs were also maintained without adding metals.

3.6. Analysis of metals in clams

Clams were opened and soft tissues were dissected out using clean stainless steel scissors and forceps into digestive gland, gill and whole soft tissue. The tissue samples were gently washed with double distilled water.

3.7. Digestion procedure

The samples were wet-oxidised using the procedure given by FAO (1975), UNEP (1990 & 1993). The samples were weighed and 1 gm of it was taken in a 100 ml spoutless beaker. 10 ml of a mixture of conc. HNO_3 and H_2O_2 (1:1) was added per 1 gm of the sample. The whole thing was kept as such for an hour and after that it was placed inside a sandbath and gently heated. Temperature was kept at 60°C and the digestion time was nearly 8 hrs and is continued until the volume got

Table 3(i) : Metal concentration and physico-chemical parameters of the water used for the bioaccumulation experiment.

Metal	Conc. (ppm)	length of the clam	Salinity (ppt)	Temperature	Dissolved Oxygen	pH
Cu2+	0.1	28-35	10 ± 2	29.5 ± .25	>90%	7.8
	0.25	28-35	10 ± 2	29.5 ± .25	>90%	7.8
Pb2+	0.3	28-35	10 ± 2	29.5 ± .25	>90%	7.8
	0.6	28-35	10 ± 2	29.5 ± .25	>90%	7.8

reduced to half. Blank was prepared in the same way without the samples. Samples were made upto 25 ml using double distilled water in a volumetric flask.

3.8. Instrumental determination of metals

The Copper and Lead concentrations were determined in a Perkin Elmer 2380 Model Atomic Absorption Spectrophotometer using air acetylene flame. The samples were aspirated directly into the flame and the corresponding readings were noted. Standards of metal solutions were prepared using AAS standard solutions (1000 ppm) from Merck. The metal concentration is calculated using the relationship

$$\text{Element in microgram / g} = \frac{(C)(v)(df)}{w}$$

Where C is the conc. of the element in the sample solution in µg/l; v is the volume of the undiluted sample solution in ml. w is the sample weight in gms and df is the dilution factor.

$$df = \frac{\text{vol. of diluted sample solution in ml}}{\text{vol. of aliquot taken for dilution in ml}}$$

3.9. Analysis of metals in the sediment

The samples were dry-oxidised using the procedure given by

FAO(1975), UNEP, (1990) & (1993).

Reagents used

1. Nacl-30 gms in 100ml metal free distilled water.

2. Acid solution-10 vol. of Perchloric acid ($HClO_4$ sp.gr. 1.67) and 2 vol. of conc. HNO_3 (sp.gr. 1.40).

3. Hydroxylammonium chloride 50/100 ml $HONH_3Cl$

Samples collected from the sites were air dried and ground using a pestle and mortar and sieved through a mesh (No. 85 meshes/inch). 1 gm of the sample was taken in 100 ml conical flask and to it added 3 drops of Nacl and 8 ml of acid solution. It was mixed well and kept for digestion in a sand bath at 70-80°C for 12 hrs.

When the digestion was completed (which was indicated by the light yellowish colour of the filtrate) the samples were taken out, allowed to cool and added 3 drops of hydroxylammonium chloride. Then it was mixed well and made upto 50 ml with double distilled water in a volumetric flask.

3.10. Instrumental determination of metals

Copper, Lead & Cadmium in the samples collected from

different sites were determined in a Perkin Elmer 2380 Model AAS in the similar way described earlier for the biological sample and metal concentration is calculated using the relationship explained earlier.

3.11. HISTOLOGY

This part deals with the histopathological effects on the gills and digestive gland of *Villorita cyprinoides* exposed to sublethal concentrations of copper and lead.

3.11.1. Procedure of micro slide preparation

After termination of the exposure period, the animals were dissected out and gills and digestive tubules were fixed in Bouin's fixative for 24 hrs.

The following procedure was used for the preparation.

1. Washed overnight in running water.

2. The tissues were treated with saturated solution of lithium carbonate in 70 % alcohol (ethyl) to remove yellow colour of picric acid.

3. After softening, the tissues were stored in fresh 70 % alcohol. In

this stage, the tissues can be stored till further processing.

4. Tissues dehydrated by transferring them sequentially to 80, 90 & 95 % alcohol- 2hr each.

5. Transferred to absolute (100%) alcohol (2 changes) for 1hr each.

6. The tissues were placed in 1:1 mixtures of absolute alcohol and xylene for 30 min.

7. The tissues were cleared in xylene until the tissues became translucent.

8. Tissues transferred to a mixture of xylene and paraffin wax and left overnight.

9. The tissues were infiltered in 2-3 changes of molten paraffin wax of melting point 60-62°C for 1 hr each.

10. The tissues were embedded in paraffin wax of melting point 60-62°C .

The blocks were trimmed and sections of 5mm thickness were cut with a rotary microtome.

3.11.2. STAINING TECHNIQUE FOLLOWED WITH TRIPLE MALLORY STAIN

1. Deparaffinised and hydrated slides to water as $Hgcl_2$ is absent from fixative, sections treated with saturated aquous mercuric chloride (mordant), plus 5% glacial acetic acid for 10 mins.

2. Washed, treated with Lugol's Iodine and sodium thiosulphate

3. Washed and rinsed in distilled water

4. Stained in Mallory I : 15 secs.

5. Rinsed in distilled water : 10 sec.

6. Treated in Phosphomolybdic acid : 1-5 min.

7. Stained in Mallory II : 2 min.

8. Rinsed in distilled water

9. Differentiated aniline blue in 90% alcohol for 5 sec.

10. Dehydrated in absolute alcohol (2changes): 1 min. each

11. Cleared in xylene

12. Mounted in D.P.X.

3.12. PLANIMETRY

Planimetric studies were made using computer assisted image analysis. The image analysis system consists of a high resolution CCD colour camera (COHU mod.8215.2000) mounted on a light microscope. The image is displayed on a computer screen and captured using an image analysis program (Scion image 1.44) with PC software on a Compaq (Pentium 166 Mhz) computer. The program allows the user to acquire, display, edit, enhance, analyze and print the images. Images were viewed using a 10-40x objective. Five images of digestive tubules were randomly taken from each duplicate section from each animal. All images for each metal were captured in one session during which the microscope illumination (powered by a stabilized DC supply) and camera setting were kept constant. The digital image consisted of an 8 bit, 320 X 240 matrix of picture elements (pixels), where each pixel consisted of a number between 0 and 255 representing the intensity of transmitted light (or gray level) at a point. Stored images were later analyzed to determine the Epithelial thickness (ET), Luminal Radius (LR) and Tubular Radius (TR) (fig.II).

3.13. Statistical Analysis

Differences in MET (Mean Epithelial Thickness), MLR (Mean Luminal Radius) & MTR (Mean Tubular Radius) values in the digestive tubules of clams exposed to different concentrations of metals (Cu^{2+} & Pb^{2+})

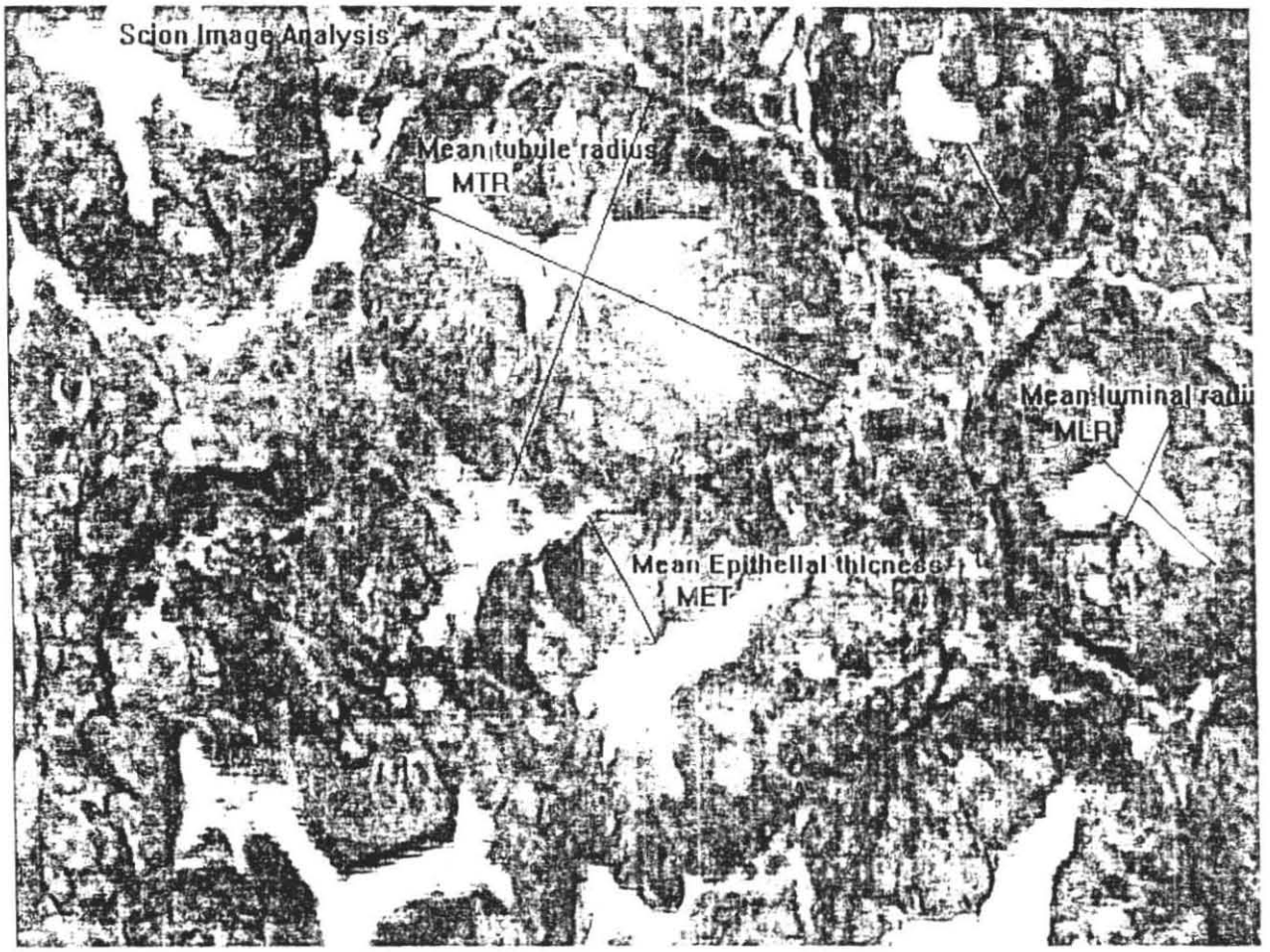

Fig II : Shows the Parameters selected for the Planimetric study using Scion Image Analysis.

and also those from different sites were tested by analysis of variance (ANOVA). Differences in the above mentioned parameters were assessed using the Fisher's LSD Multiple Comparison Test, Scheff's S & Dunnett One-Tailed Tests. Findings were considered significant at a > 0.05.

3.14. ULTRASTRUCTURE

In this section, studies were conducted to assess the pathological changes at the cellular level of *V.cyprinoides* exposed to cu (0.25ppm) and lead (0.6ppm) for 21 days. After termination of the exposure period, the animals were cut open and the digestive gland was dissected out and fixed in primary fixative i.e 3% buffered glutaraldehyde solution for 3hrs at 4°c, following immersion fixation technique.

The following time-chart was adopted for making tissue blocks.

1. Tissues were trimmed into small pieces of $1mm^3$ size.

2. Tissues were washed thrice in cacodylate buffer (0.1M sodium cacodylate) with a duration of 15 minutes(5 min. each).

3. Post fixation of the tissues was done using 1% Osmium tetroxide at 4°c for 2hrs.

4. Osmium tetroxide was drained out and the tissues were washed in several changes of fresh buffer solution. 15min. each time. This is to remove excess osmium tetroxide that could form a black precipitate in the tissue.

5. The tissues were dehydrated at 4°c in ascending series of acetone by immersing for 10 min. each in 30%, 50%, 70%, 90% and finally 100% giving two changes of 10 minutes duration at room temperature.

6. The infiltration of the tissues was done using different concentrations of acetone and spurr's resin(Spurr,1969) in the ratio 3 : 1, 1 : 1 and 1 : 3 for 1hour each.

7. Embedding done in Spurr embedding media keeping the plastic capsules in the cintex incubator at 70°c for 9hrs.

From the polymerized blocks ultrathin sections were cut in the LKB ultratome NOVA and stained in uranyl acetate and lead citrate, double stage staining to enhance the contrast.

The ultrathin sections mounted on the grids and observed. The image was recorded in the Hitachi H 600 Transmission Electron Microscope.

4. RESULTS

4.1. ECOLOGY

Both physical and chemical parameters of the water collected fortnightly from the four stations, viz: Station 1(Panangad), Station 2(Chambakara), Station 3(Konthuruthy) and Station 5(Chembu) and that water collected on a seasonal basis from other four stations Station 4(Vaduthala), Station 6(Pallipuram), Station 7(Thannirmukkom) and Station 8(Muhamma) were analysed. The monthly variation of physical parameters (Temperature, PH of sediment & water, salinity and dissolved oxygen) and chemical parameters (phosphate, nitrite and nitrate) of the water collected from Station 1(Panangad), Station 2(Chambakara), Station 3(Konthuruthy) and Station 5(Chembu) are depicted in fig.3,4,5 & 6 respectively. The seasonal variation of the same physical and chemical parameters of the water collected from Station 4(Vaduthala), Station 6(Pallipuram), Station 7(Thannirmukkom) and Station 8(Muhamma) are depicted in fig.16 & 17 respectively.

During the monsoon months (June-Sep) the presence of phosphate and nitrate in the water was higher compared to the other months, for all the stations. However, nitrite did not show any significant seasonal dependence, with relatively low values during all the months. But at Station 2 (Chambakara) the nitrite values were relatively higher and showed a tendency to increase during monsoon months. Temperature and

pH remained almost the same during all the months at all the stations. During monsoon months salinity values tended to decrease at all stations. No significant variation could be observed in the case of dissolved oxygen. It was (see following section of results) seen that copper and cadmium accumulation increased during monsoon months which coincides with the slight increase noticed in the concentration of the phosphate and nitrate and reduction of salinity during these months. Correlation analysis was conducted between metal accumulation and these parameters. But this analysis did not reveal any significant dependence of metal accumulation on these hydrological parameters.

4.2. FIELD STUDY

The statistical tests applied, viz. Fisher's Protected LSD & Scheffes'S, revealed a significant variation in the concentration of metals among the four stations. The variation in the metal concentrations in the sediment and in the whole soft tissue and the digestive glands of clams at the four stations are discussed in detail in the following stations.

4.2.1. Metals in sediment

The mean concentration of copper, lead and cadmium in the sediment at the four stations are showed in table.4(i). The mean copper concentration was highest at station 5 (Chembu, 26.050 µg/g dry weight)

Fig:3(a) Monthly variation of physical parameters of water collected from station 1.

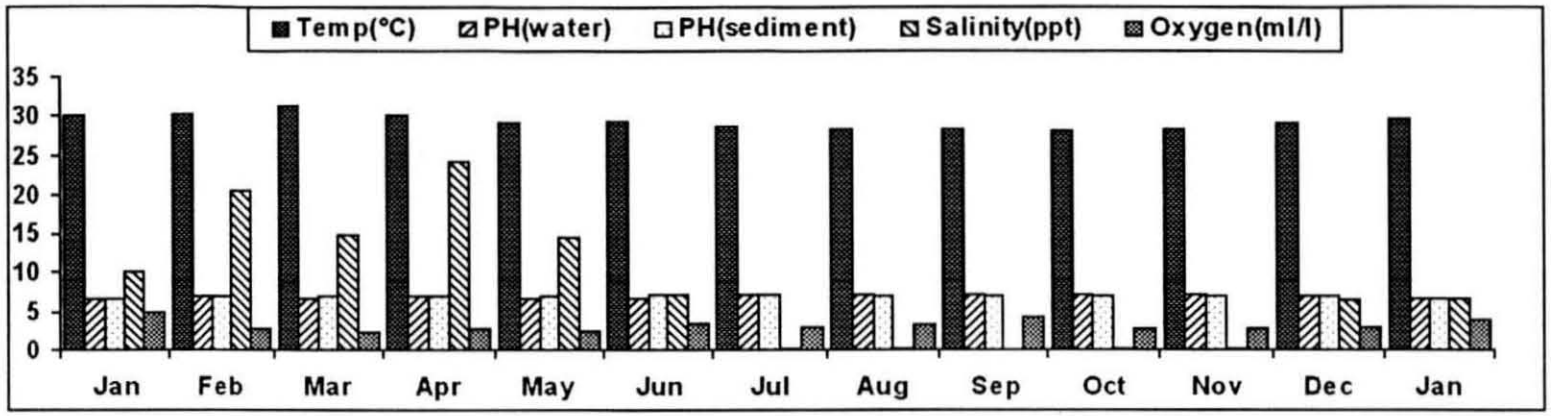

Fig:3(b) Monthly variation of nutrients in the water collected from station 1.

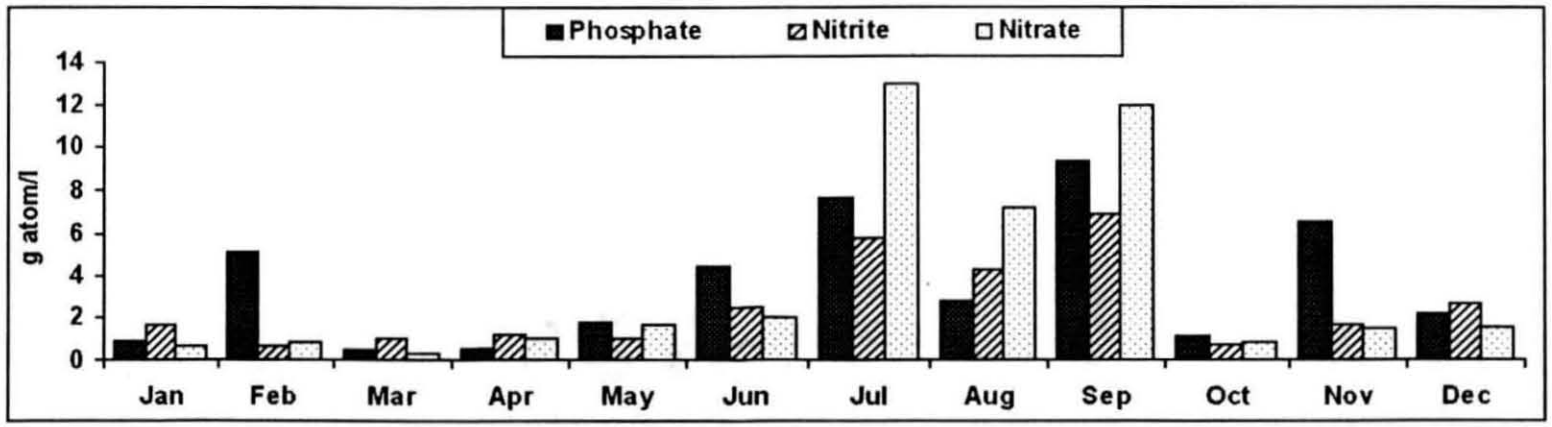

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Fig:4(a) Monthly variation of physical parameters of water collected from station 2.

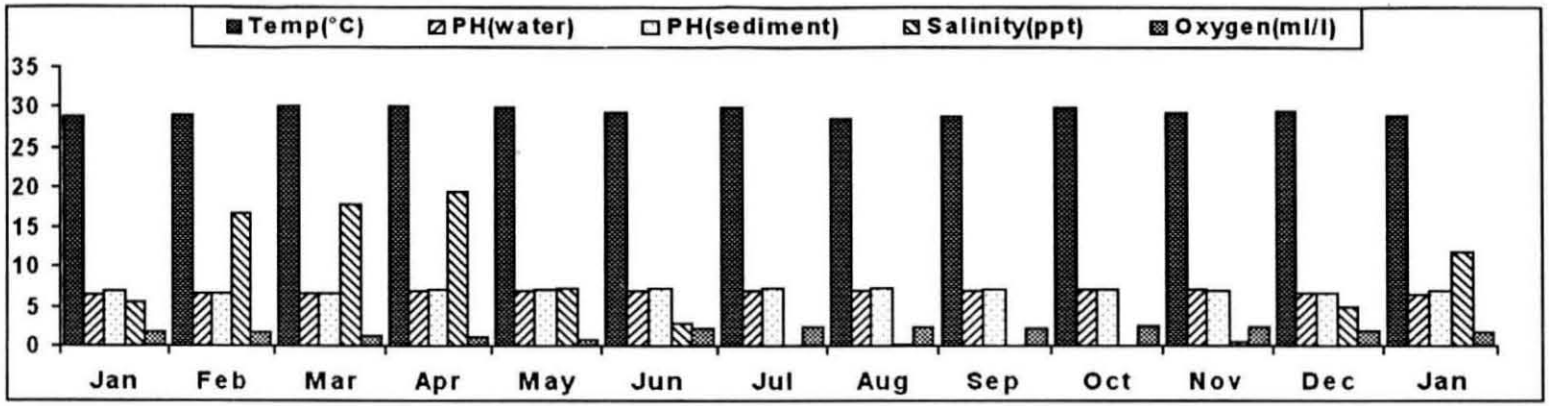


Fig :4(b) Monthly variation of nutrients in the water collected from station 2.

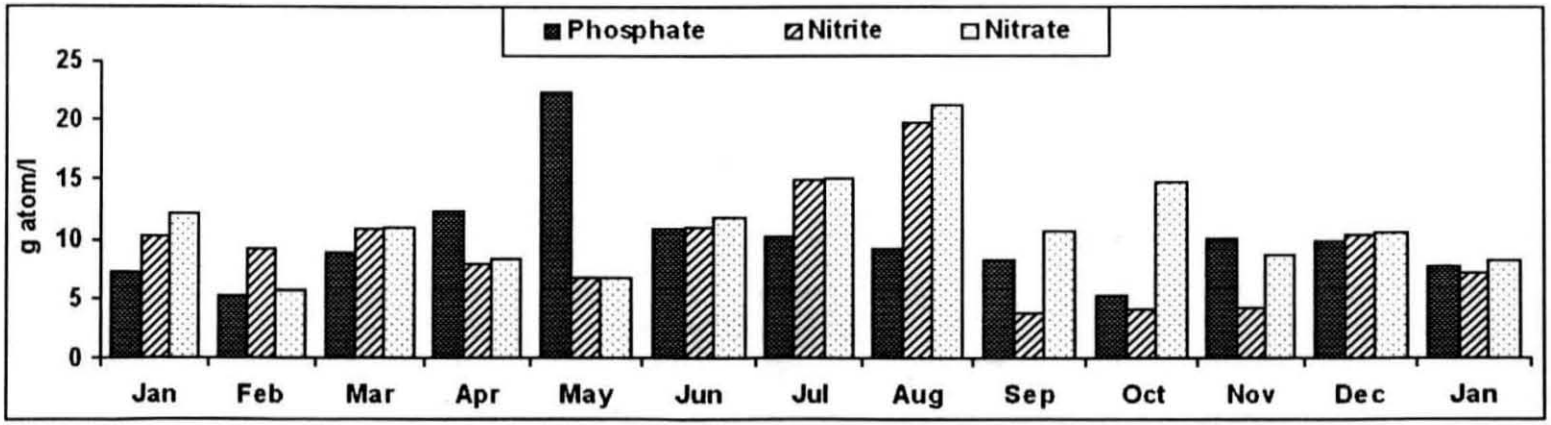


Fig: 5(a) Monthly variation of physical parameters of water collected from station 3.

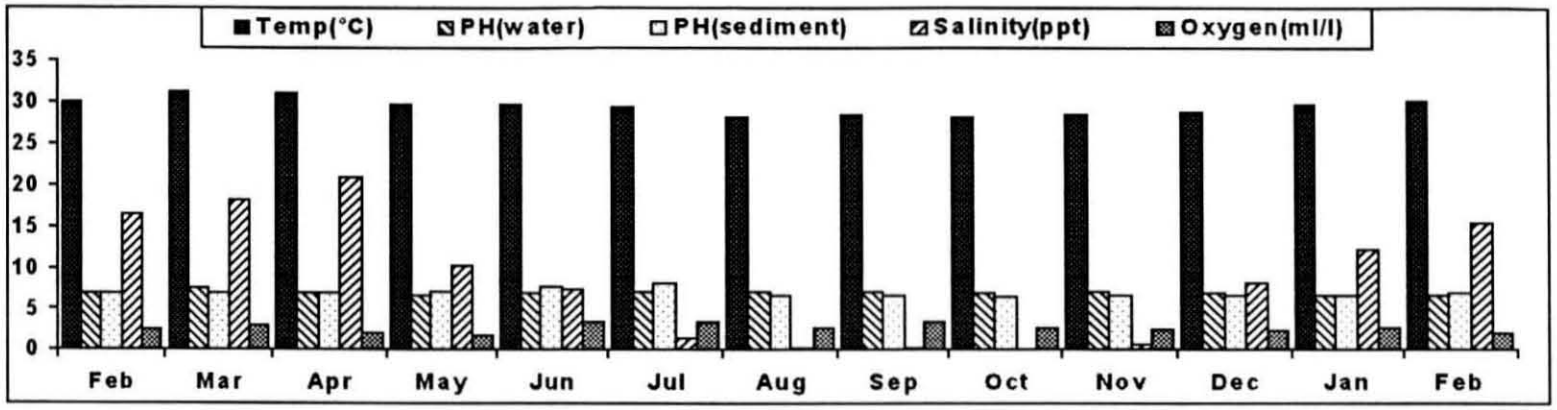


Fig: 5(b) Monthly variation of nutrients in the water collected from station 3.

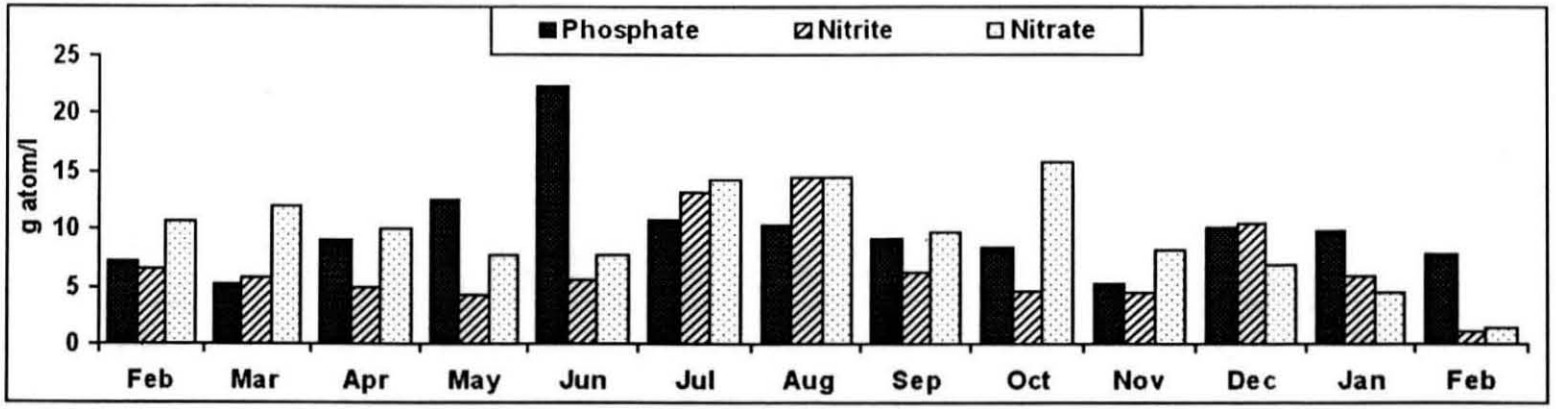


Fig: 6(a) Monthly variation of physical parameters of water collected from station 5.

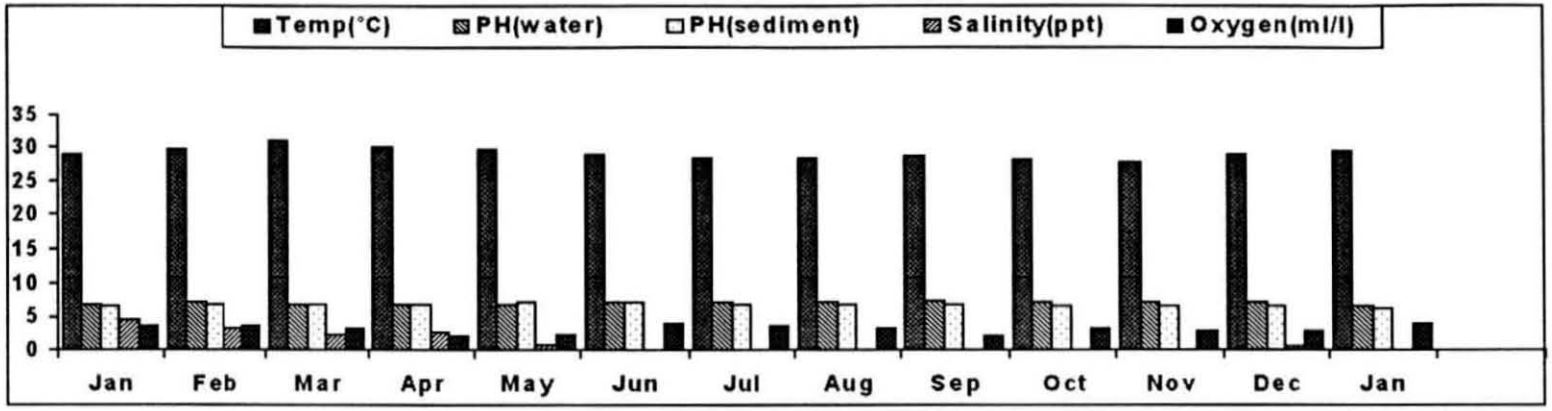
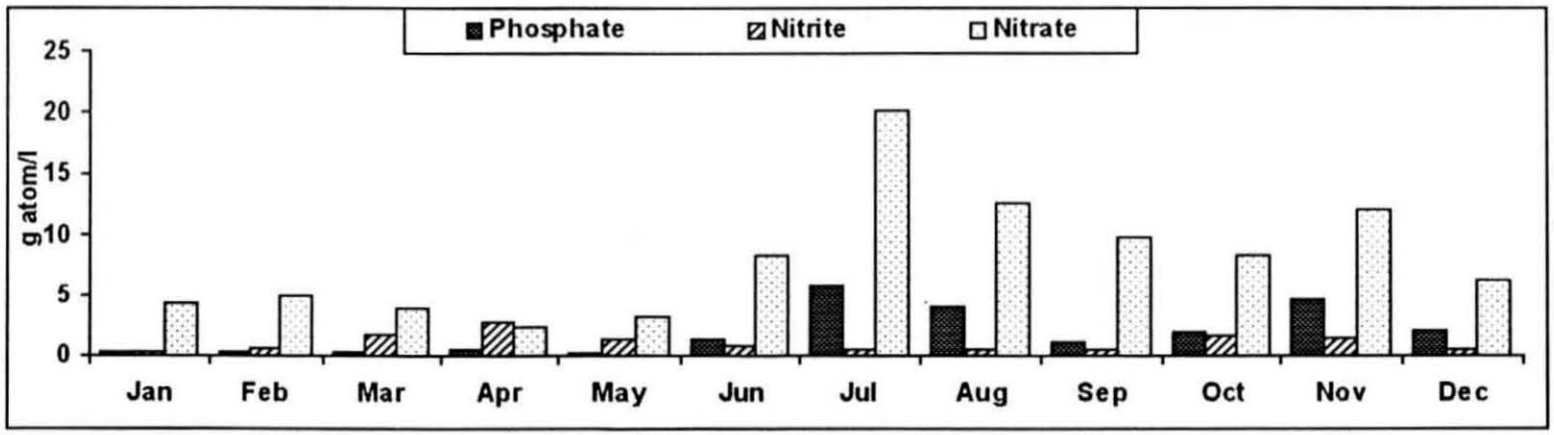


Fig: 6(b) Monthly variation of nutrients in the water collected from station 5.



followed by station 2 (Chambakara, 13.242 μg/g dry weight), station 1(Panangad, 7.910 μg/g dry weight and station 3 (Konthuruthy, 4.287 μg/g dry weight). The mean cadmium concentration was highest at station 5 (27.367 μg/g dry weight) followed by station 2 (17.858 μg/g dry weight), station 1 (12.583 μg/g dry weight) and station 3 (6.815 μg/g dry weight). The mean lead concentration was also highest at station 5 (1.583 μg/g dry weight) followed by station 1 (1.258 μg/g dry weight), station 2 (0.789 μg/g dry weight) and station 3 (0.465 μg/g dry weight).

The monthly variation of copper concentration in the sediment at the four stations are depicted in fig.7. At station 2 the highest value was 60.65 μg/g dry weight and the lowest value was 5.0 μg/g dry weight observed in October and July respectively. The highest value for copper concentration at station 5 was 60.35 μg/g dry weight observed in November and the lowest value was 8.75 μg/g dry weight observed in January. At station 1 the highest value was 13.65 μg/g dry weight and the lowest value was 5.0 μg/g dry weight observed in December and June respectively. At station 3 the highest value was 9.3 μg/g dry weight and the lowest value was 2.0 μg/g dry weight observed in November and February respectively.

In fig.8, the monthly variation of cadmium concentration in the sediment at the four stations are given. At station 1 the highest value was 6.25 μg/g dry weight and the lowest value was 0.25 μg/g dry weight observed in February and August respectively. At station 5 the highest value for cadmium concentration was 3.475μg/g dry weight observed in

Table 4(i) : Mean values of metals obtained for the sediments from four sites (µg/gm)

Station	Copper	Cadmium	Lead
Chembu	26.05	27.367	1.583
Panangad	7.910	12.583	1.258
Chambakara	13.242	17.858	0.789
Konthuruthy	4.287	6.815	0.465

Table 4(ii). Mean values of metals obtained for the whole soft tissue of clams from four sites (µg/gm).

Station	Copper	Cadmium	Lead
Chembu	2.494	0.7	0.299
Panangad	2.434	0.787	0.661
Chambakara	2.508	0.759	0.466
Konthuruthy	2.999	0.797	0.673

Table 4(iii). Mean values of metals obtained for the digestive gland of clams from four sites (µg/gm).

Station	Copper	Cadmium	Lead
Chembu	3.396	0.66	0.683
Panangad	2.775	0.819	0.737
Chambakara	2.774	0.895	0.737
Konthuruthy	3.243	0.735	0.793

February and the lowest value was 0.825 µg/g dry weight observed in January. At station 2 highest value was 1.525 µg/g dry weight and the lowest value was 0.375 µg/g dry weight observed in May and November repectively. At station 3 the highest value was 0.825 µg/g dry weight and the lowest value was 0 observed in March and February respectively.

The monthly variation of lead concentration in the sediment at the four stations are depicted in fig.9. The highest value for lead concentration at station 5 was 42.5 µg/g dry weight observed in September and the lowest value was 6.75 µg/g dry weight observed in January. At station 2 the highest value was 29.75 µg/g dry weight and the lowest value was 9.5 µg/g dry weight observed in February and July respectively. At station 1 the highest value was 18.25 µg/g dry weight and the lowest value was 6.1 µg/g dry weight observed in March and October respectively. At station 3 the highest value was 10.25 µg/g dry weight and the lowest value was 4 mg/g dry weight observed in May and February respectively.

4.2.2. Metals in soft tissues of clam (*Villorita cyprinoides*)

The mean concentration of copper, cadmium and lead in the whole soft tissue of clams collected from the four stations are given in table.4(ii). Mean copper concentration was highest at station 3 (2.999 µg/ g wet weight) followed by station 2 (2.508 µg/g wet weight), station 5(2.494 µg/g wet weight) and station 1 (2.434 µg/g wet weight). Mean

Fig: 7 Monthly variation of copper in sediment collected from four stations.

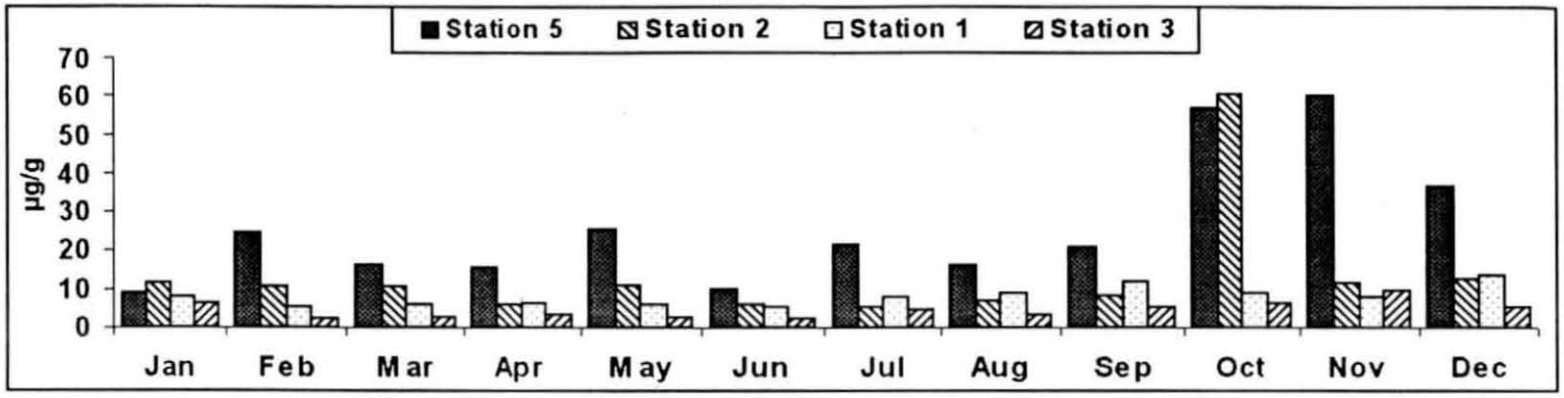


Fig: 8 Monthly variation of cadmium in sediment collected from four stations.

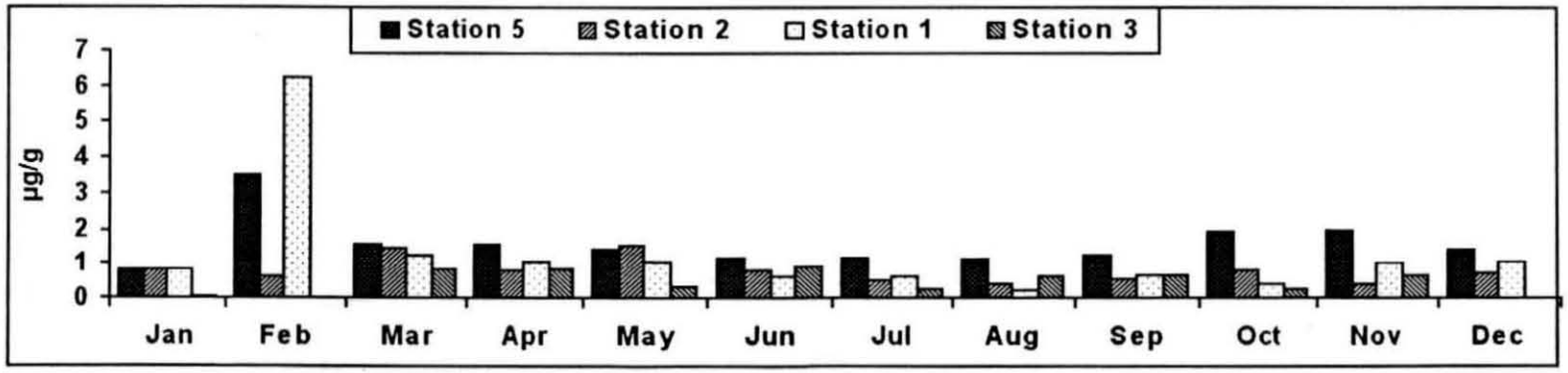
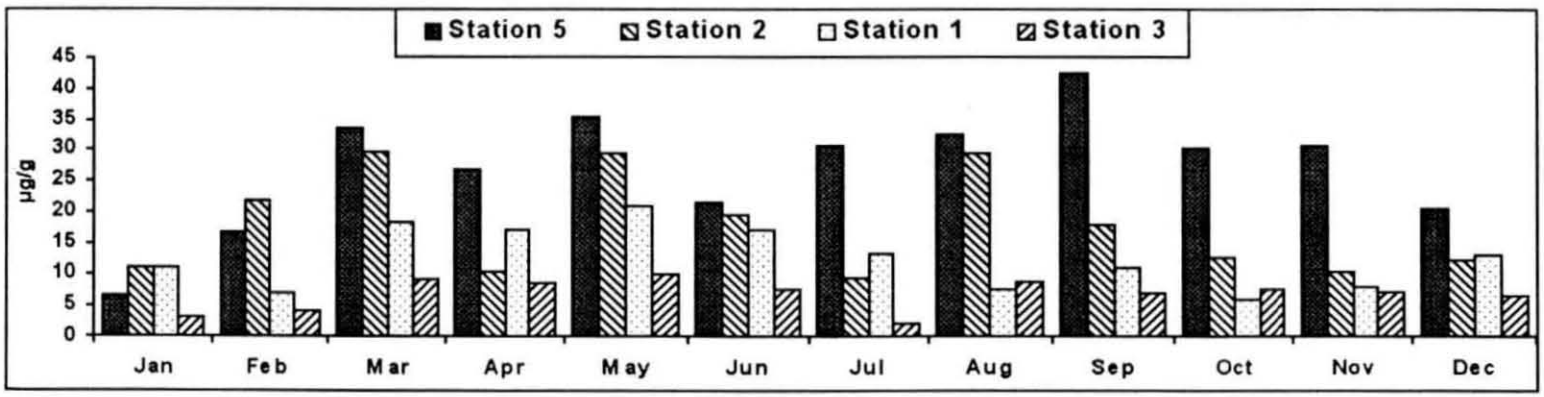


Fig: 9 Monthly variation of lead in sediment collected from four stations.



cadmium concentration was highest at station 3 (0.797 µg/g dry weight) followed by station 1 (0.787 µg/g wet weight), station 2 (0.759 µg/g wet weight) and station 5 (0.700 µg/g wet weight). Mean lead concentration was highest at station 3 (0.673 µg/g wet weight) followed by station 1 (0.661 µg/g wet weight), station 2 (0.466 µg/g wet weight) and station 5 (0.299 µg/g wet weight).

Copper:

The monthly variation of copper concentration in the whole soft tissue of clams from the four stations are depicted in fig.10. At station 3 the highest value was 3.84 µg/g wet weight and the lowest value was 1.4 µg/g wet weight observed in September and December respectively. At station 2 the highest value was 3.76 µg/g wet weight and the lowest value was 1.41 µg/g wet weight observed in October and February respectively. At station 1 the highest value was 3.675 µg/g wet weight and the lowest value was 1.413 µg/ g wet weight observed in July and January respectively. The highest value for copper concentration at station 5 was 3.25 µg/g wet weight observed in July and the lowest value was 1.0 µg/g wet weight observed in December.

Cadmium:

In fig.11. the monthly variation of cadmium concentration in the whole soft tissue of clams at the four stations are shown. At station 3 the highest value was 2.2 µg/g wet weight and the lowest value was 0.275 µg/g wet weight observed in May and September respectively. At station 1 the highest value was 1.25 µg/g wet weight and lowest value

was 0.448 µg/g wet weight observed in April and January respectively. At station 5 the highest value was 1.3 µg/g wet weight observed in April and the lowest value was 0.25 µg/g wet weight observed in October. At station 2 the highest value was 1 µg/g wet weight observed during the months of April, May and August and the lowest value was 0.4 µg/g wet weight observed in December.

Lead:

The monthly variation of lead concentration in the whole soft tissue of clams from the four stations are depicted in fig.12. At station 3 the highest value was 2 µg/g wet weight observed in April and the lowest value was 0 observed during the months of March,June and October. At station 2 very low values were observed during most of the months except during July and August when the observed lead concentration was about 1.75 & 1 µg/g wet weight respectively. At station 1 the highest value was 1.75 µg/g wet weight observed in October and the lowest value was 0 observed during the months of July, August, November and December. The highest value for lead concentration at station 5 was 0.85 µg/g wet weight observed in December and the lowest value was 0 observed during the months of April, May, June, July, September and October.

The mean concentration of copper, cadmium and lead in the digestive glands of clams collected from the four stations are shown in table.4(iii). The mean copper concentration was highest at station 5 (3.396 µg/g wet weight) followed by station 3 (3.243 µg/g wet weight), station 1

Fig: 10 Monthly variation of copper in the whole soft tissue of clams collected from four stations.

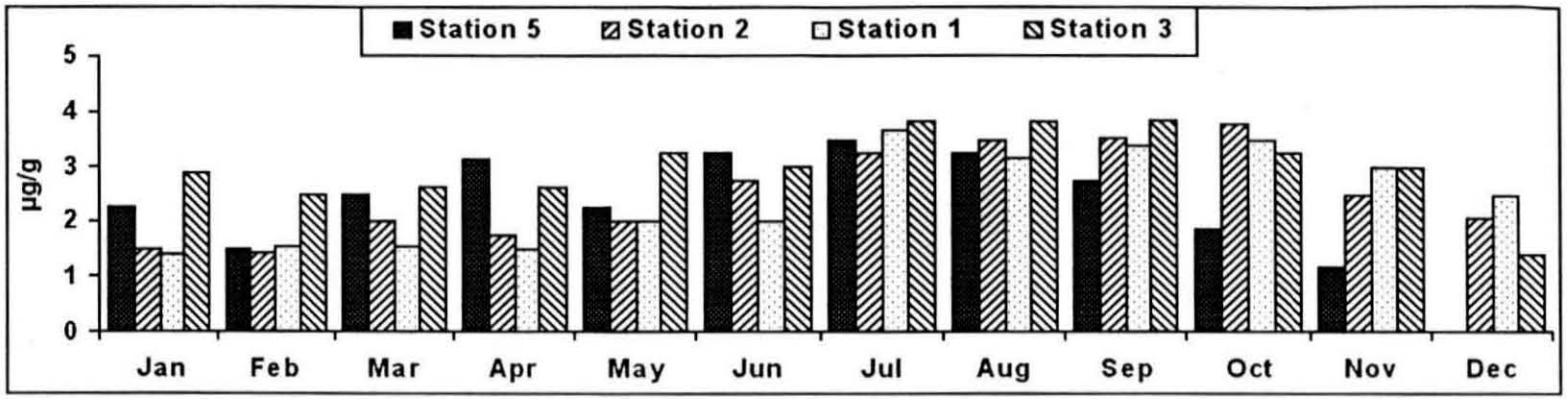


Fig: 11 Monthly variation of cadmium in the whole soft tissue of clams collected from four stations.

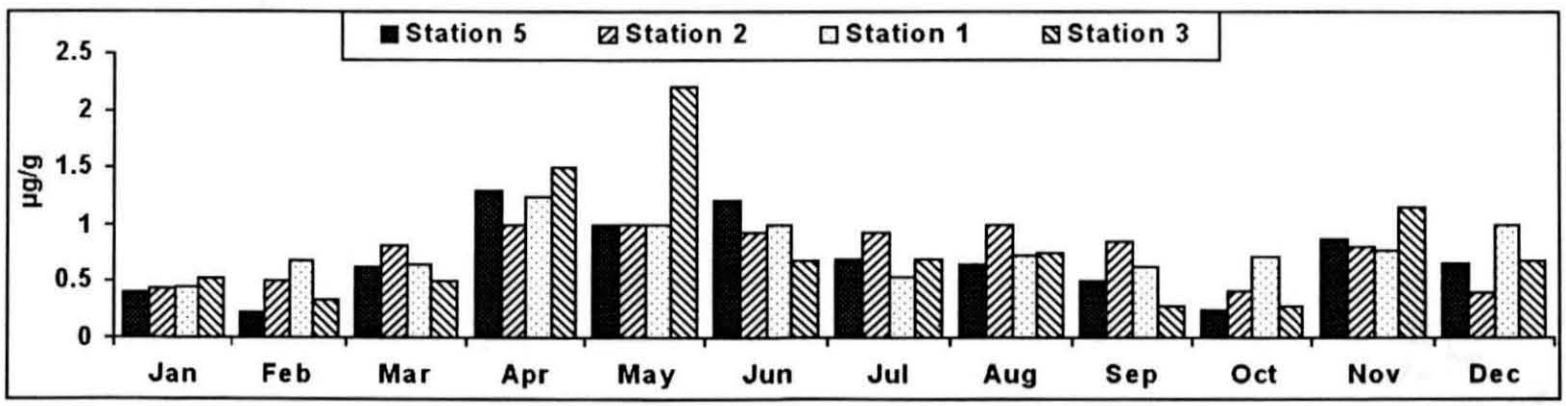
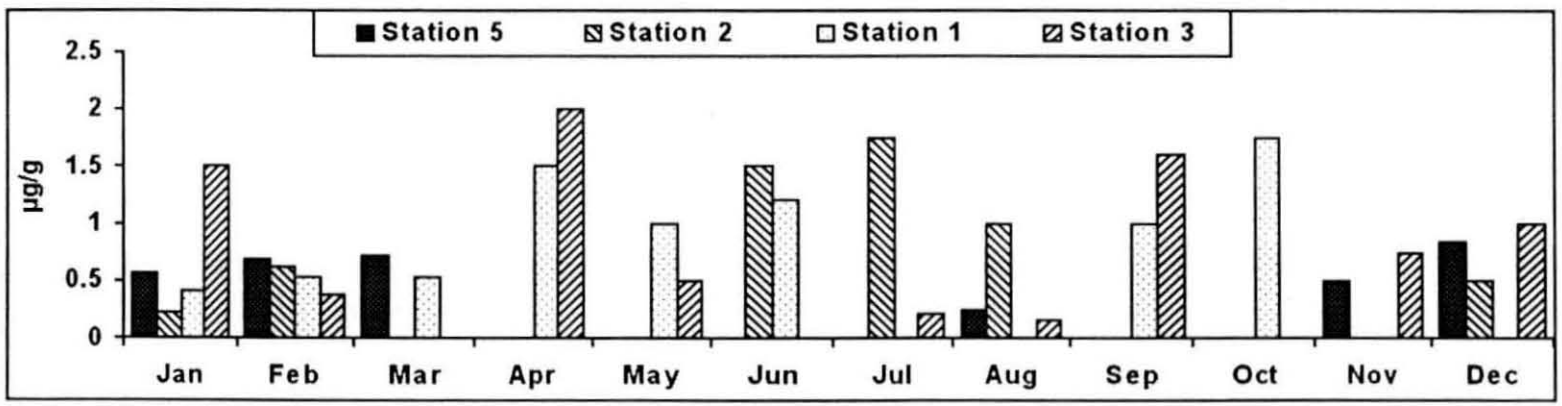


Fig: 12 Monthly variation of lead in the whole soft tissue of clams collected from four stations.



(2.775 μg/g wet weight) and station 2 (2.774 μg/g wet weight). The mean cadmium concentration was highest at station 2 (0.895 μg/g wet weight) followed by station1 (0.819 μg/g wet weight), station 3 (0.735 μg/g wet weight) and station 5 (0.664 μg/g wet weight). The mean lead concentration was highest at station 3 (0.793 μg/g wet weight) followed by station 2 and station1 (same value of 0.737 μg/g wet weight) and station 5 (0.683 μg/g wet weight).

The monthly variation of copper concentration in the digestive glands of clams at the four stations are depicted in fig.13. The highest value of copper concentration at station 5 was 5.0 μg/g wet weight observed in July and the lowest value was 2.5 μg/g wet weight which was observed during the months of January, February and October. At station 3 the highest value was 3.95 μg/g wet weight and the lowest value was 2 μg/g wet weight observed in February and December respectively. At station 2 the highest value was 3.8 μg/g wet weight and the lowest value was 1.93 μg/g wet weight observed in September and February respectively. At station 1 the highest value was 3.8 μg/g wet weight and the lowest value was 2 μg/g wet weight observed in October and December respectively.

In fig.14. the monthly variation of cadmium concentration in the digestive glands of clams at the four stations are shown. At station 2 the highest value was 1.8 μg/g wet weight and the lowest value was 0.35 μg/g wet weight observed in April and November respectively. At station 3 the highest value was 1.5 μg/g wet weight and the lowest value was

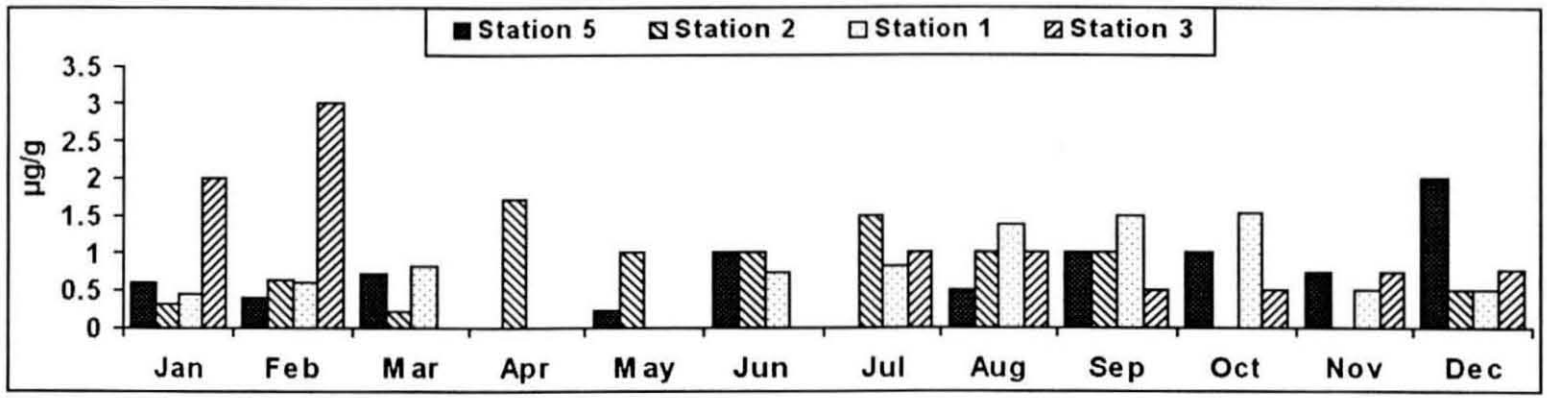
0.38 μg/g wet weight observed in April and October respectively. At Panangad the highest value was 1.23 μg/g wet weight and the lowest value was 0.475 μg/g wet weight observed in April and November respectively. The highet value for cadmium concentration at station 5 was 1 μg/g wet weight observed in April and November and the lowest value was 0.225 μg/g wet weight observed in October.

The monthly variation of lead concentration in the digestive glands of clams at the four stations are depicted in fig.15. At station 3 the highest value was 3 μg/g wet weight observed in February and the lowest value was 0 observed during March,April, May and June. The highest value of lead concentration at station 5 was 2 μg/g wet weight observed in December and the lowest value was 0 observed in July. At station 2 the highest value was 1.7 μg/g wet weight and the lowest value was 0.32 μg/g wet weight observed in April and January respectively. At station 1 the highest value was 1.5 μg/g wet weight and the lowest value was 0 observed in October and April respectively.

In general, copper concentration in the whole soft tissue of clams collected from all the four stations exhibited high values during monsoon and post-monsoon months. Comparatively low values were observed during pre-monsoon months. Cadmium concentrations were also high in the post-monsoon months at all the four stations. However, lead concentrations did not showed any such specific pattern.

The concentration of all the three metals in the digestive glands

Fig: 15 Monthly variation of lead in the digestive glands of clams collected from four stations.



of clams were higher in comparison to the metal concentration in the whole soft tissue at all the four stations. Copper concentration in the digestive glands were higher during the monsoon months whereas cadmium concentration was higher during the pre-monsoon months. But lead concentrations did not show any marked seasonal pattern.

4.2.3. Metal accumulation dependence on clam size

The mean concentration of the three metals in the digestive glands of the two size groups of clams (small: 20-30mm; large: 30-40mm) from station 1 and 5 are shown in table 4(iv). The mean copper concentration was found to be higher in the larger size group at both the stations. At station 1 the cadmium and lead concentrations were also found to be higher in larger size group. But at station 5 there was a slight decrease in both the cadmium and lead concentrations with an increase in clam size. Significant difference in the accumulation of copper and cadmium (p-value<0.0001) by different size groups was obtained using one sample t-test. However, lead accumulation was not much significantly different between two size groups.

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4.2.4. SEASONAL STUDY

As mentioned in section 3.1.4., four stations, station 4 (Vaduthala), station 6 (Pallipuram), station 7 (Thannirmukkom) and station

Table 4(iv) : Comparison of mean values of metals in the digestive gland of clams of two size groups (μg/gm).

	Size	Copper	Cadmium	Lead
Panangad	20-30mm	2.57	0.71	0.32
	30-40mm	2.78	0.82	0.74
Chembu	20-30	2.75	0.686	0.82
	30-40	3.396	0.664	0.683

8 (Muhamma) were selected for the seasonal study. Samples (Sediment, water and clam) were collected during the three seasons (pre-monsoon, monsoon & post-monsoon). The results of which are given in the following section. The seasonal vatiations in the hydrological parameters aat the four stations are depicted in figures 16 & 17.

Station 4(Vaduthala)

The seasonal variation in metal concentration at Vaduthala in sediment, whole soft tissue and digestive glands of clams are shown in fig.18(a), 18(b) & 18(c). In sediment, the concentration of copper was highest (10 µg/g dry weight) during the post-monsoon season. High lead concentrations (9.5 & 10.2 µg/g dry weight) were observed during pre-monsoon and post-monsoon seasons. Cadmium concentrations were significantly low compared to that of copper and lead for all the seasons.

Analysis of whole soft tissue of clams revealed highest concentration of copper during the post-monsoon seasons. For lead and cadmium the values were very low during the entire period of study.

Highest value of copper concentrations in the digestive glands of clams were observed during the post-monsoon season. Very low values of lead and cadmium were obtained for all the seasons.

Station 6(Pallipuram)

The seasonal variation in metal concentration at Pallipuram in sediment, whole soft tissue and digestive glands of clams are shown in fig. 19(a), 19(b) & 19(c). In sediment, concentration of copper was highest (7.15 µg/g dry weight) during post-monsoon seasons. Lead value was highest (15.5 µg/g dry weight) during pre-monsoon season. But very low value was recorded during monsoon seasons. Cadmium concentration was vey low during the entire period of study.

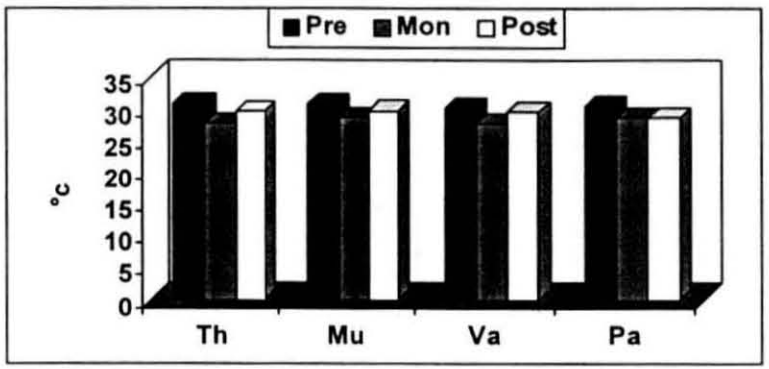
Analysis of whole soft tissue revealed comparatively high concentration (2µg/g wet weight) of copper during the monsoon season. Lead and cadmium concentrations were low during all the seasons.

High concentration of copper was recorded in the digestive gland during the monsoon and post- monsoon seasons. Very low values were recorded for cadmium and lead during the entire period of study.

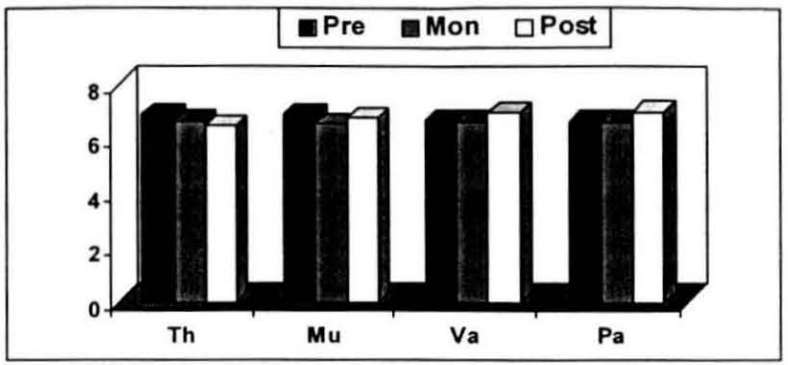
Station 7(Thannirmukkom)

The seasonal variation in metal concentrations at station 7 in sediment, whole soft tissue and digestive gland of clams are shown in fig. 20(a), 20(b) & 20(c) respectively. In sediment, concentration of copper was highest during post-monsoon season with a maximum value of 10.5 µg/g dry weight. High lead concentrations were observed during both

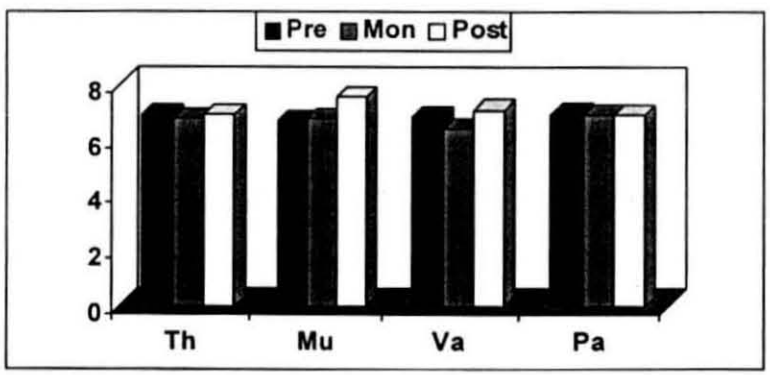
Fig. 16: Physical parameters studied at the four stations during Premonsoon, Monsoon & Post monsoon seasons



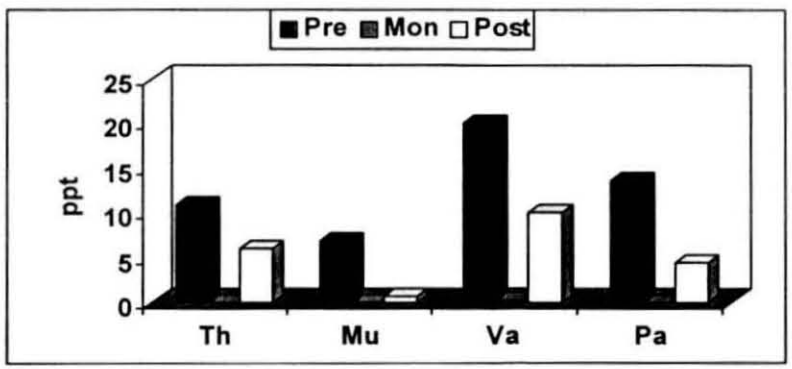
Temperature



Water PH



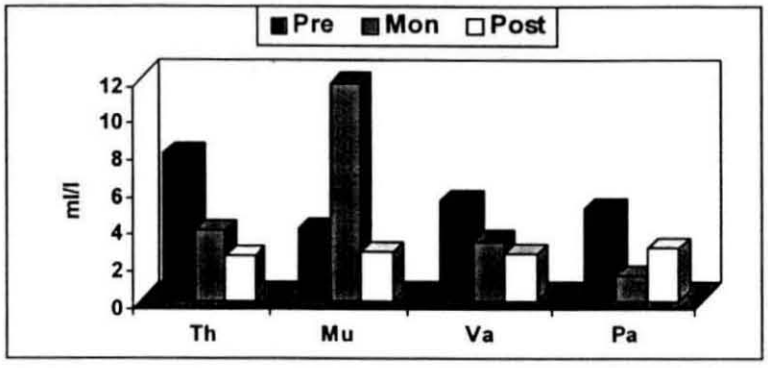
Sediment PH



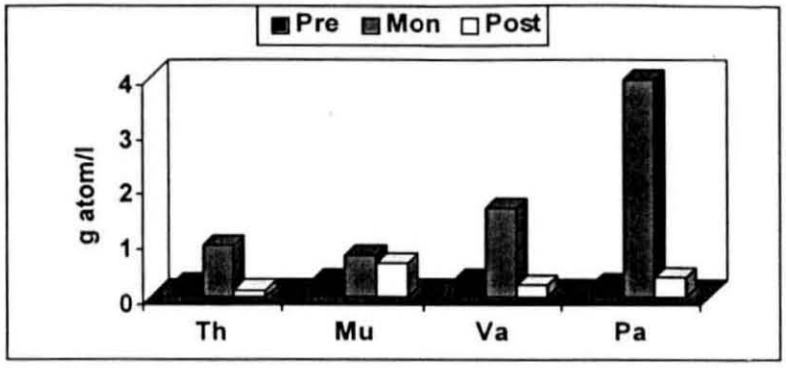
Salinity

Th - Thannirmukkom	**Va** - Vaduthala
Mu - Muhamma	**Pa** - Pallipuram

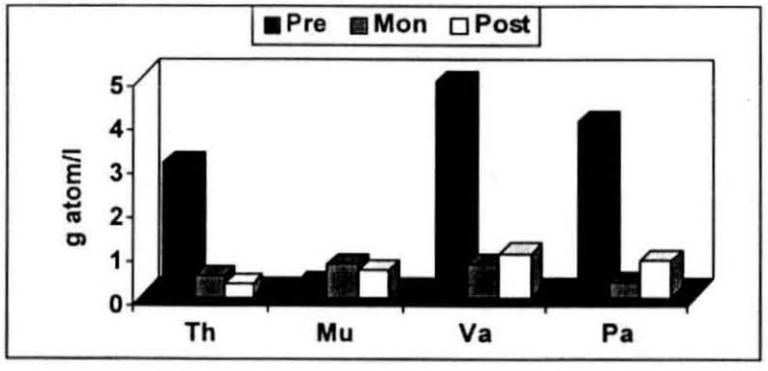
Fig. 17: Parameters studied at the four stations during Premonsoon, Monsoon & Post monsoon seasons



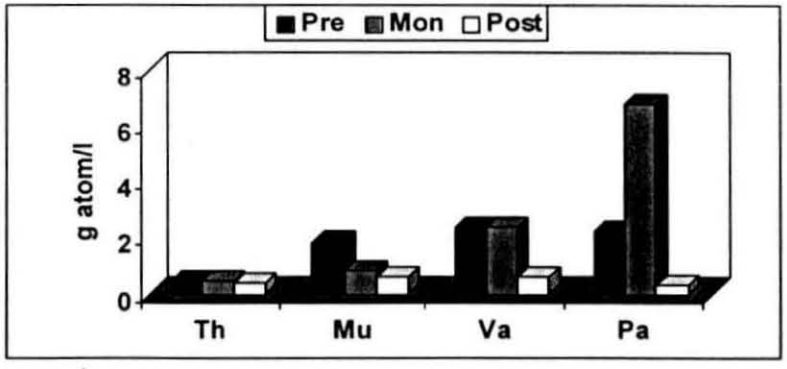
Dissolved Oxygen



Phosphate



Nitrite



Nitrate

Th - Thannirmukkom	**Va** - Vaduthala
Mu - Muhamma	**Pa** - Pallipuram

Fig. 18(a), (b) & (c): Copper, cadmium and lead concentration in the sediment, whole soft body and digestive gland of clams collected in three seasons (Pre-Monsoon, Monsoon & Post-Monsoon) respectively.

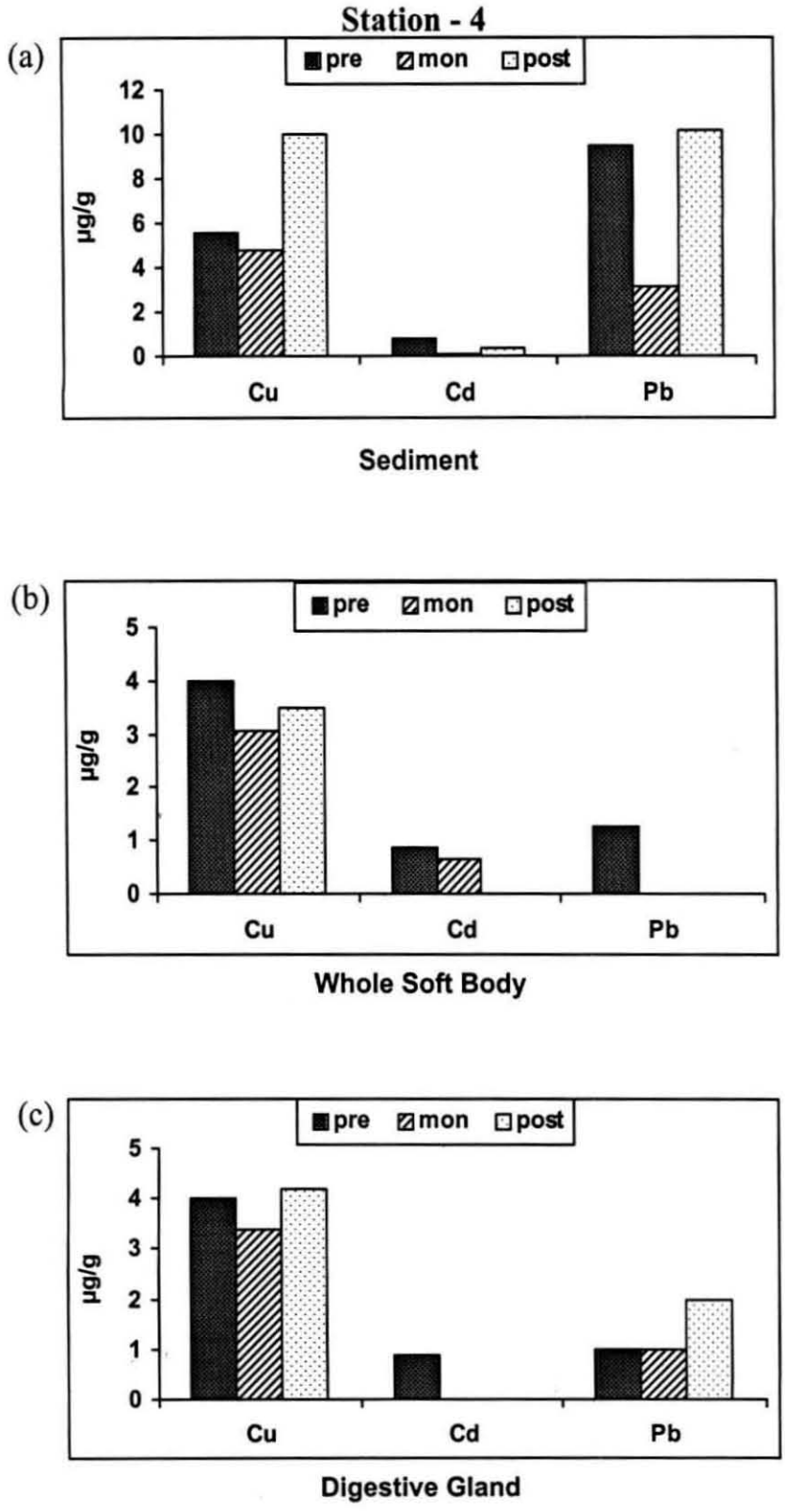
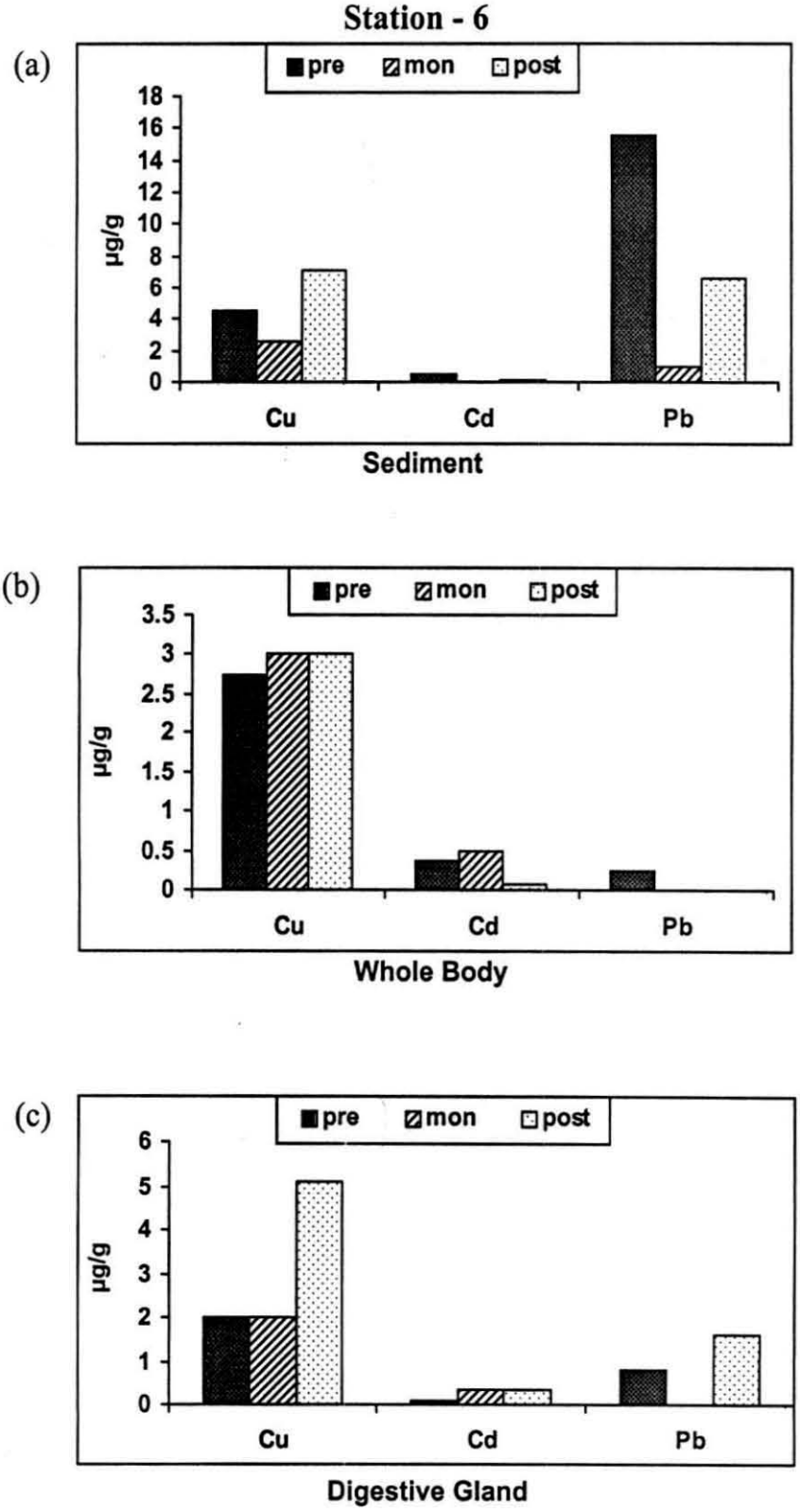


Fig. 19(a), (b) & (c): Copper, cadmium and lead concentration in the sediment, whole soft body and digestive gland of clams collected in three seasons (Pre-Monsoon, Monsoon & Post-Monsoon) respectively.



monsoon (20 µg/g dry weight) and post-monsoon (25 µg/g dry weight) seasons. The cadmium concentration was significantly lower than that of copper and lead for all the seasons with the highest value (1.4 µg/g dry weight) during the post-monsoon season.

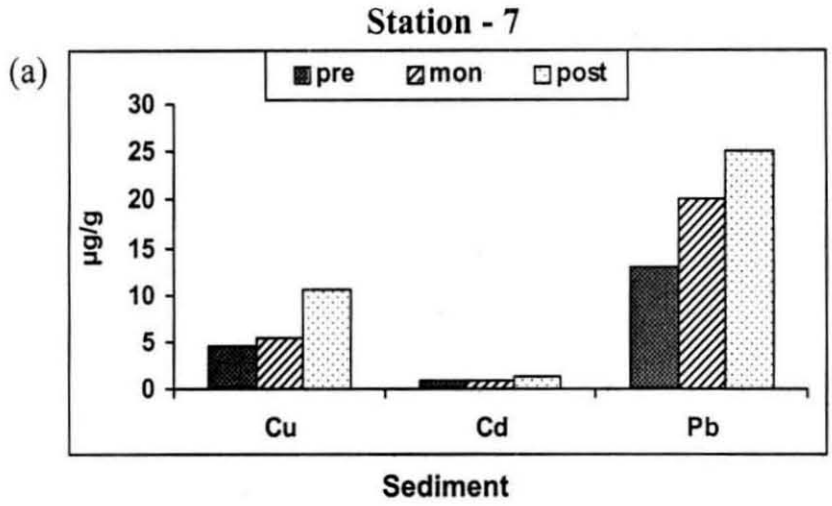
Analysis of whole soft tissue of clams also revealed highest concentration of copper during the post monsoon season (2.5 µg/g dry weight). The lead and cadmium concentrations in the soft tissue were in general very low. The cadmium values were below 0.5 µg/g wet weight during the entire period of observation. The lead values were also very low, but showed a slight increase during the post-monsoon (2.51 µg/g wet weight) season.

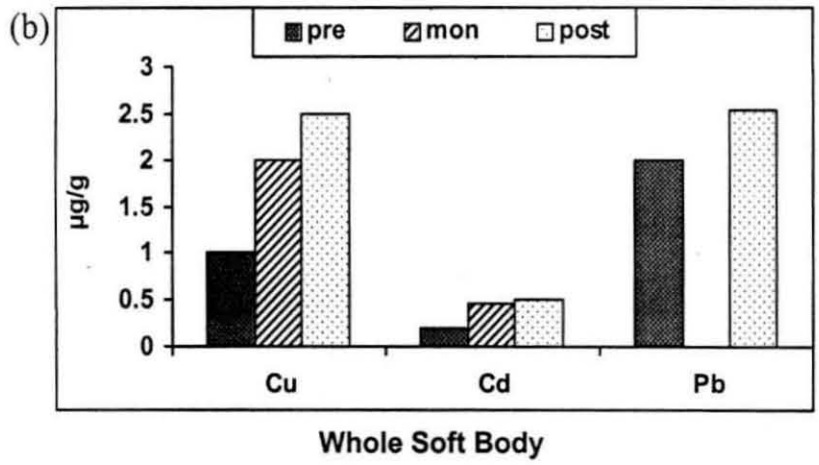
Copper concentration in the digestive glands of clams was highest (5.1 µg/g wet weight) during the post-monsoon season. Cadmium and lead concentrations in the digestive glands were very low but the lead concentrations showed an increase during the post-monsoon seasons.

Station 8(Muhamma)

The seasonal variation in metal concentration at station 8 in sediment, whole soft tissue and digestive galnd of clams are shown in fig. 21(a), 21(b) & 21(c). Analysis of sediment showed high concentration of copper (27µg/g dry weight) during the post-monsoon season. But comparatively low values (16.4 & 10 µg/g dry weight) were obtained during

Fig. 20(a), (b) & (c): Copper, cadmium and lead concentration in the sediment, whole soft body and digestive gland of clams collected in three seasons (Pre-Monsoon, Monsoon & Post-Monsoon) respectively.





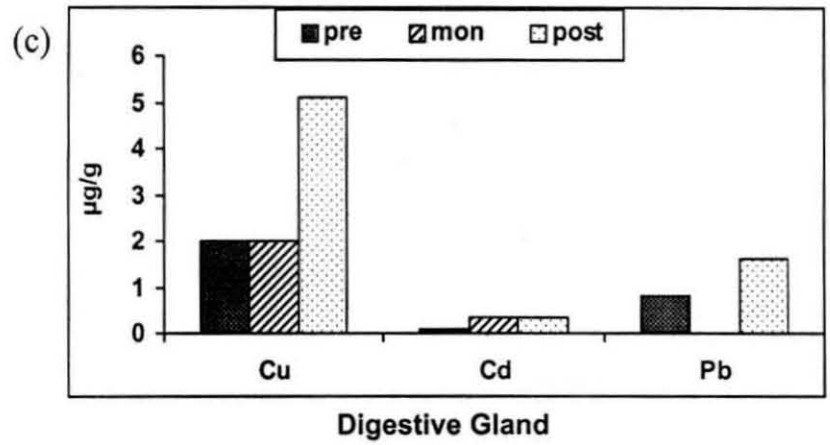
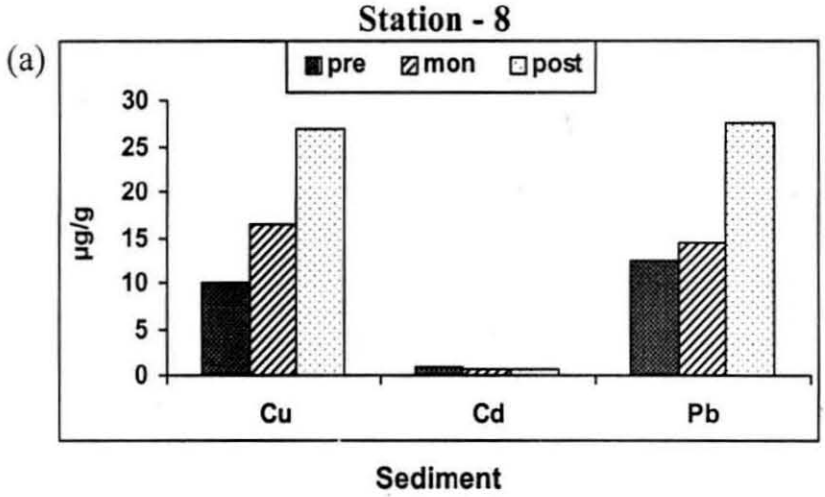
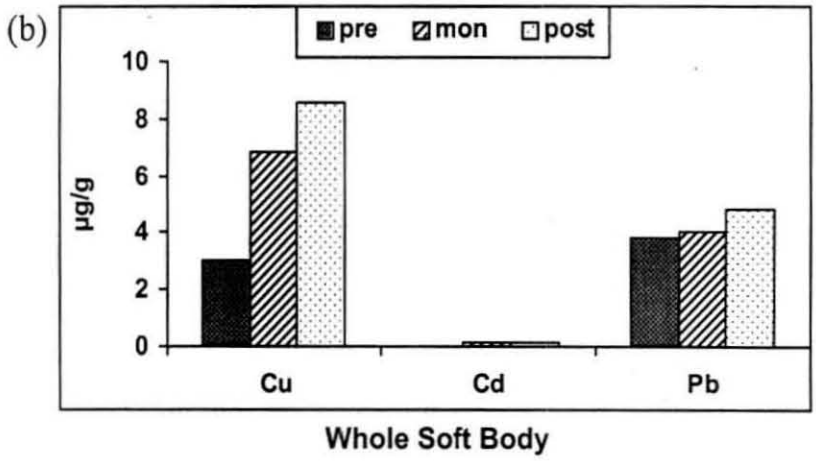
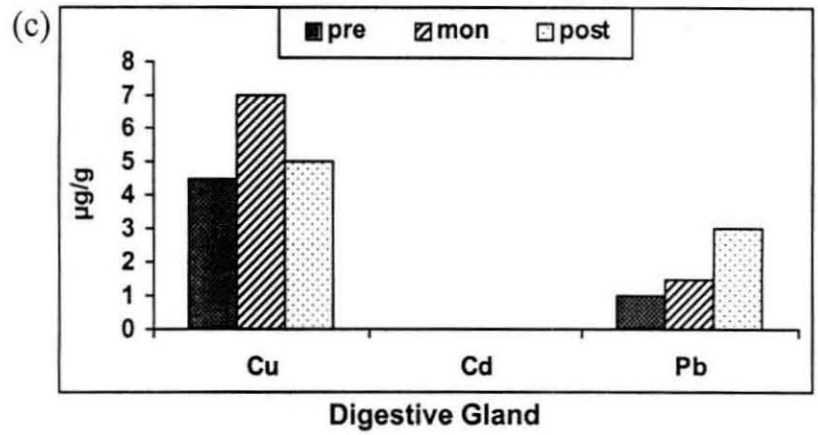


Fig. 21(a), (b) & (c): Copper, cadmium and lead concentration in the sediment, whole soft body and digestive gland of clams collected in three seasons (Pre-Monsoon, Monsoon & Post-Monsoon) respectively.







monsoon and post-monsoon seasons respectively. Cadmium concentration was very low during all the seasons. A high lead concentration (27.5 μg/g wet weight) was observed during the post-monsoon season and comparatively low values (14.55 & 12.5 μg/g dry weight) were observed during the monsoon and pre-monsoon seasons.

In whole soft tissue of the animal, the highest value of copper (8.53 μg/g wet weight) was observed during the post-monsoon season and comparatively low value (6.83 μg/g wet weight) was recorded during the monsoon season. The cadmium concentrations were very low during all the seasons. Maximum lead concentration (4.85 μg/g wet weight) was recorded during the post-monsoon season.

Analysis of digestive gland of clams showed high concentration of copper (7 μg/g wet weight) during the monsoon season. Cadmium concentrations were below the detection limits. Lead values showed a slight increase (3 μg/g wet weight) during the post-monsoon season.

4.3. ACUTE TOXICITY

The cumulative % mortality in different concentrations of copper and lead are given in table 4(v) & 4(vi) LC50 was calculated from the % mortality using US EPA Probit Analysis Program version 1.5. Results of which are given in table 4(vi), 4(vii), 4(ix) & 4(x) and fig 22 & 23.

Table 4(v) : Cumulative % mortality of *V. cyprinoides* exposed to copper for 96h

METAL	Conc. (ppm)	EXPOSURE TIME (HOURS)							
		12	24	36	48	60	72	84	96
Copper	0.5	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	20	30	50
	1.5	0	0	10	20	30	40	70	80
	2	0	0	20	30	50	60	70	80
	2.5	0	0	10	30	50	60	80	80
	3	0	10	20	40	50	80	100	100
	3.5	0	10	20	40	60	100	100	100
	4	0	20	40	60	90	100	100	100

Table 4(vi) : Cumulative % mortality of *V. cyprinoides* exposed to lead for 96h

METAL	Conc. (ppm)	EXPOSURE TIME (HOURS)							
		12	24	36	48	60	72	84	96
Lead	1.5	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	20	50
	4.5	0	0	0	0	10	20	30	50
	6	0	0	0	0	20	40	50	70
	7.5	0	0	0	10	30	50	60	80
	9	0	10	20	40	50	70	80	90
	10.5	0	20	40	60	90	100	100	100
	12	0	20	50	80	90	100	100	100

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING LC/EC VALUES
Version 1.5

Table 4(vii)

Copper

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
0.5000	10	0	0.0000	0.0000	0.0930
1.0000	10	5	0.5000	0.5000	0.3999
1.5000	10	8	0.8000	0.8000	0.6448
2.0000	10	8	0.8000	0.8000	0.7924
2.5000	10	8	0.8000	0.8000	0.8768
3.0000	10	9	0.9000	0.9000	0.9251
3.5000	10	9	0.9000	0.9000	0.9533
4.0000	10	10	1.0000	1.0000	0.9702

Chi - Square for Heterogeneity (calculated) = 4.079
Chi - Square for Heterogeneity
(tabular value at 0.05 level) = 12.592

Mu = 0.071457
Sigma = 0.281708

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.746343	0.234335	(4.287046, 5.205640)
Slope	3.549778	0.706380	(2.165274, 4.934283)

Theoretical Spontaneous Response Rate = 0.0000

Table 4(viii)

Copper

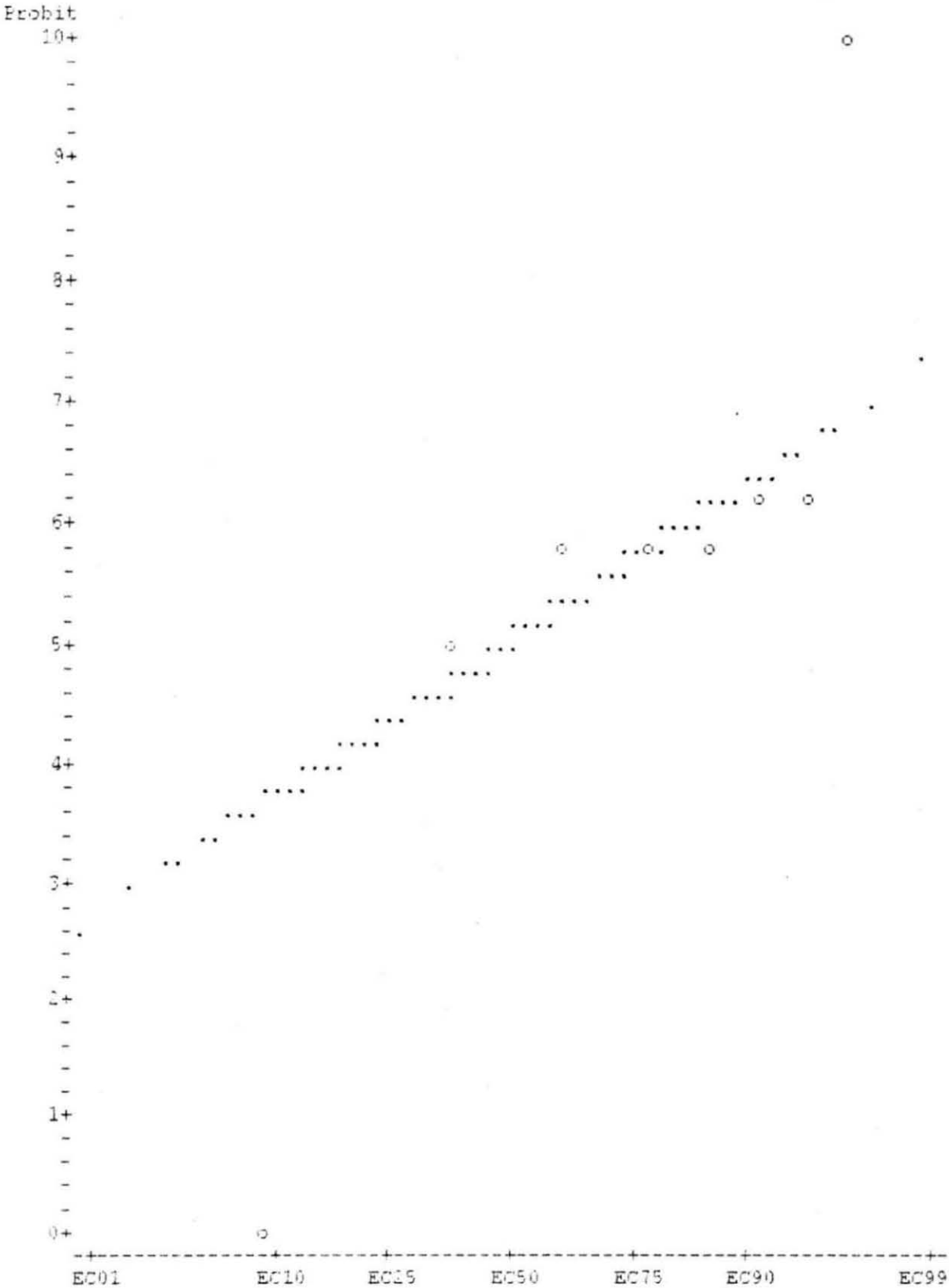
Estimated LC50 Values in ppm and Confidence Limits

Point	Exposure Conc.	95% Confidence Limits Lower	Upper
LC/EC 1.00	0.261	0.078	0.452
LC/EC 5.00	0.406	0.159	0.628
LC/EC 10.00	0.513	0.232	0.750
LC/EC 15.00	0.602	0.299	0.848
LC/EC 50.00	**1.179**	**0.832**	**1.486**
LC/EC 85.00	2.309	1.830	3.299
LC/EC 90.00	2.707	2.110	4.164
LC/EC 95.00	3.426	2.568	5.965
LC/EC 99.00	5.331	3.625	11.986

Fig : 22

Copper

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE



EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING LC50 VALUES
Version 1.5

96 hr LC50 values for Lead

Table 4(ix)

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
1.5000	10	0	0.0000	0.0000	0.0421
3.0000	10	5	0.5000	0.5000	0.3050
4.5000	10	5	0.5000	0.5000	0.5798
6.0000	10	7	0.7000	0.7000	0.7600
7.5000	10	8	0.8000	0.8000	0.8639
9.0000	10	9	0.9000	0.9000	0.9219
10.5000	10	10	1.0000	1.0000	0.9543
12.0000	10	10	1.0000	1.0000	0.9728

Chi - Square for Heterogeneity (calculated) = 3.865
Chi - Square for Heterogeneity
(tabular value at 0.05 level) = 12.592

Mu = 0.603355
Sigma = 0.247450

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	2.561713	0.591223	(1.402916, 3.720510)
Slope	4.041218	0.785084	(2.502454, 5.579982)

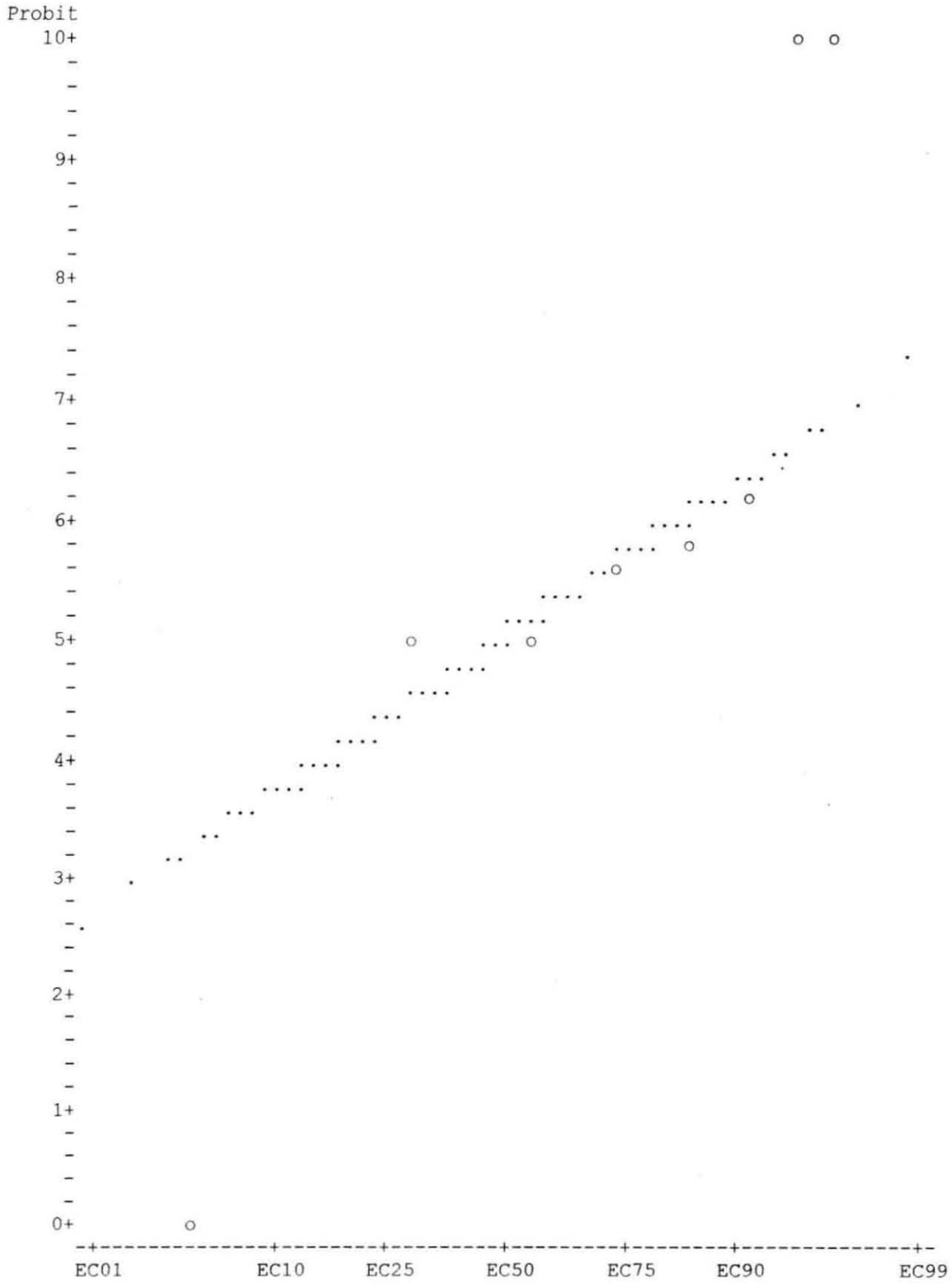
Theoretical Spontaneous Response Rate = 0.0000

Table 4(x) **Estimated LC50 Values and Confidence Limits for Lead**

Point	Exposure Conc.	95% Confidence Limits Lower	Upper
LC/EC 1.00	1.066	0.389	1.707
LC/EC 5.00	1.572	0.720	2.284
LC/EC 10.00	1.933	0.998	2.676
LC/EC 15.00	2.223	1.241	2.983
LC/EC 50.00	**4.012**	**2.992**	**4.925**
LC/EC 85.00	7.241	5.875	9.985
LC/EC 90.00	8.327	6.653	12.224
LC/EC 95.00	10.242	7.906	16.694
LC/EC 99.00	15.101	10.720	30.531

Fig : 23

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE FOR LEAD



For clams at 96h the highest concentration of copper at which mortality was not observed and the lowest concentration at which 100 % mortality was observed were 0.5 and 0.3 ppm respectively. The 96h LC 50 for copper was 1.179 ppm.

For lead, at 96 h the highest concentration at which mortality was not observed and the lowest concentration at which 100 % mortality was observed were 1.5 and 9.0 ppm respectively. 96h LC50 for lead was 4.0 ppm. In this study, the animal was, more sensitive to copper than lead.

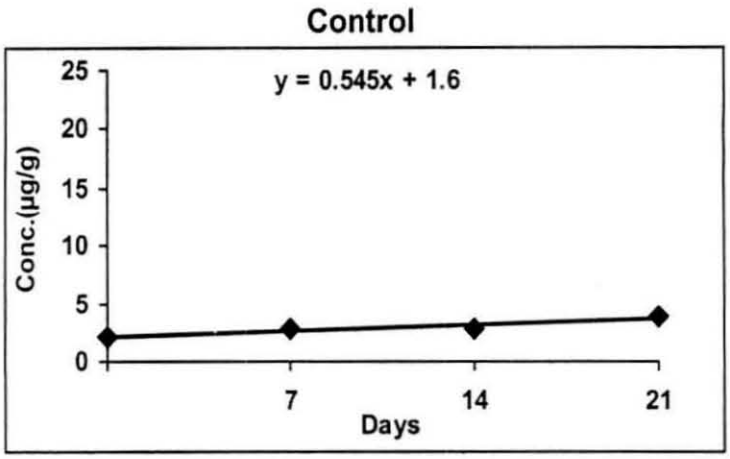
4.4. Sub-lethal Studies

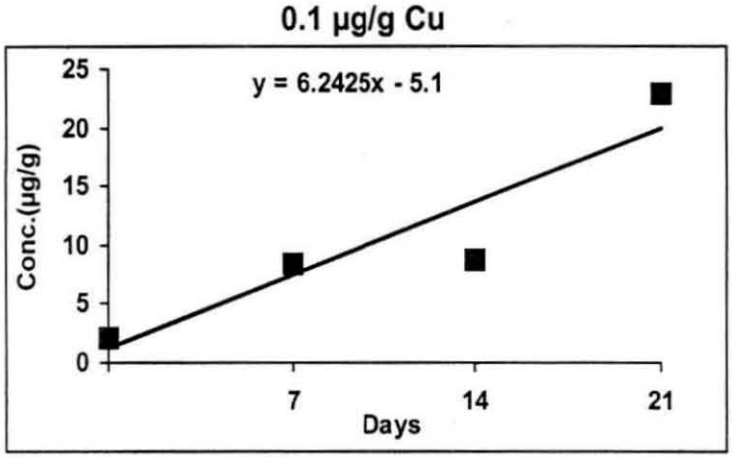
The variation of metal (copper and lead) accumulation with time in the digestive glands, gills and whole soft tissue of control specimen and test specimens are depicted in figures 24 to 29.

A steady increase with time was observed in the accumulation of copper in the digestive gland, gills and whole soft tissue in both the concentrations tested (see fig.24, 25 & 26). In both the concentrations maximum accumulation occurs in the digestive glands and gills indicating the major role played by these organs in metal accumulation. The copper concentration in the digestive glands were slightly higher in comparison to the metal accumulation in gills.

In the case of lead also, a similar steady increase with time

Fig. 24 : The linear relationship between time and concentration (copper) in the whole soft tissue of clams.





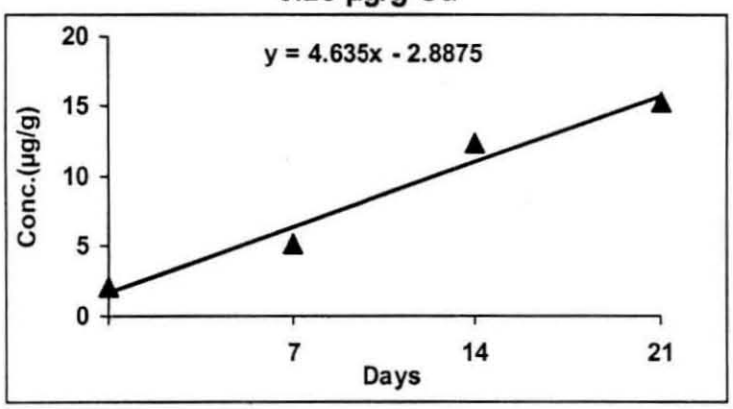
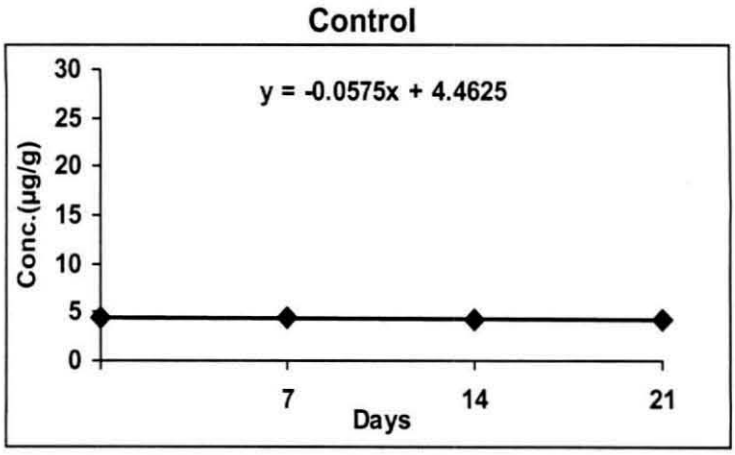
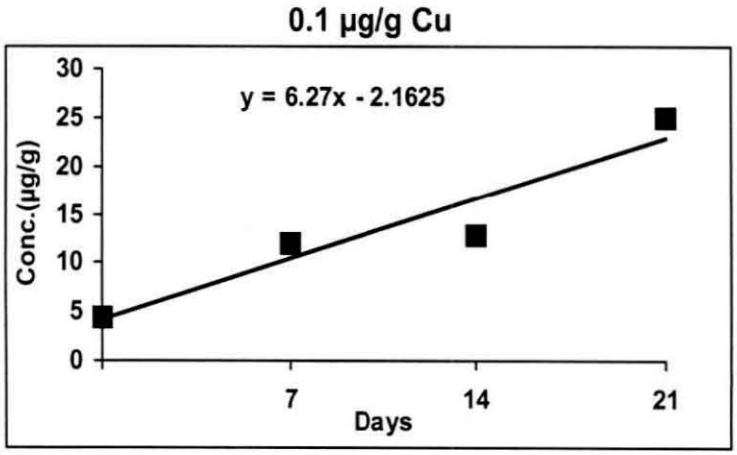


Fig. 25 : The linear relationship between time and concentration (copper) in the digestive gland of clams.





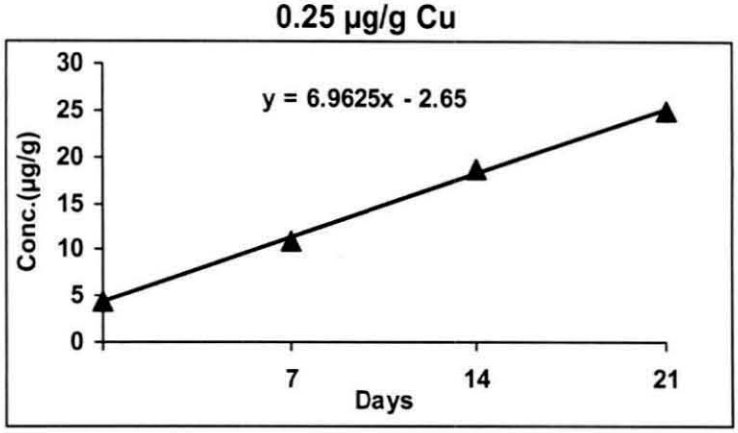
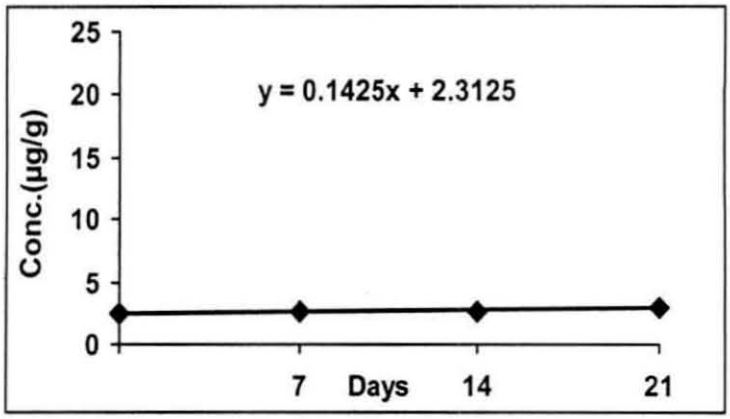
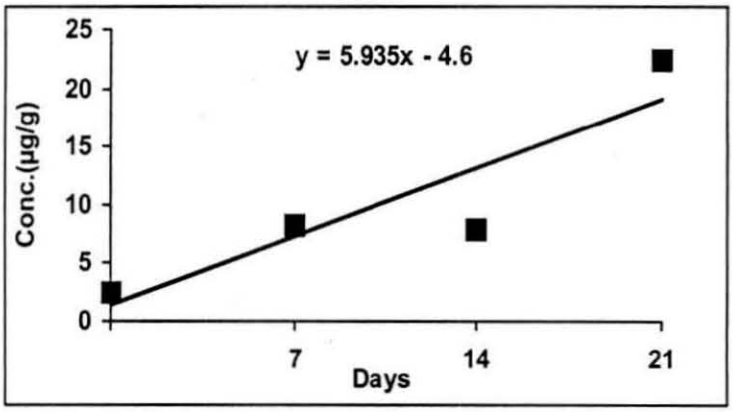


Fig. 26 : The linear relationship between time and concentration (copper) in the gills of clams.



0.1 µg/g Cu



0.25 µg/g Cu

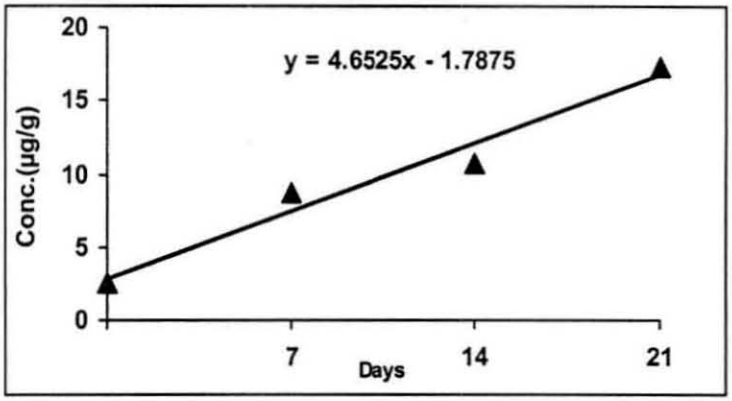
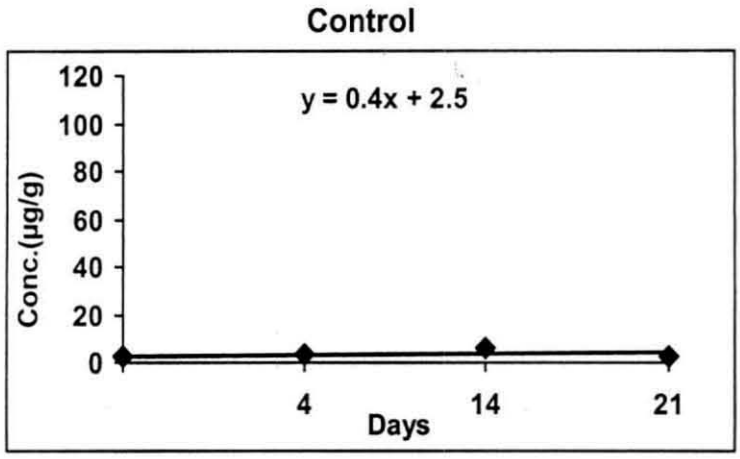
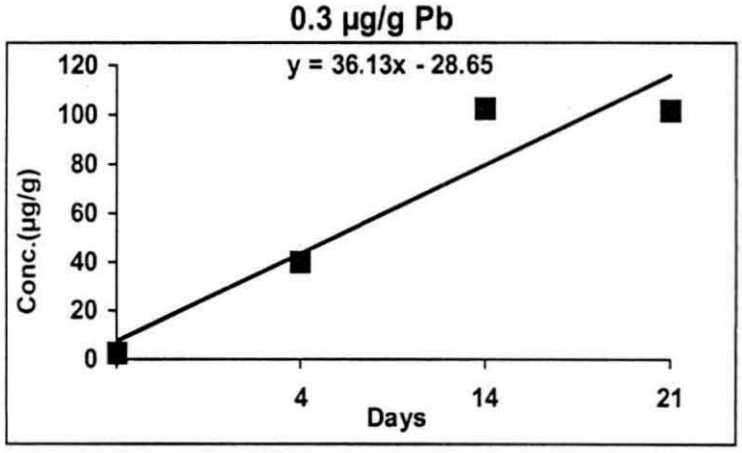


Fig. 27 : The linear relationship between time and concentration (lead) in the whole soft tissue of clams.





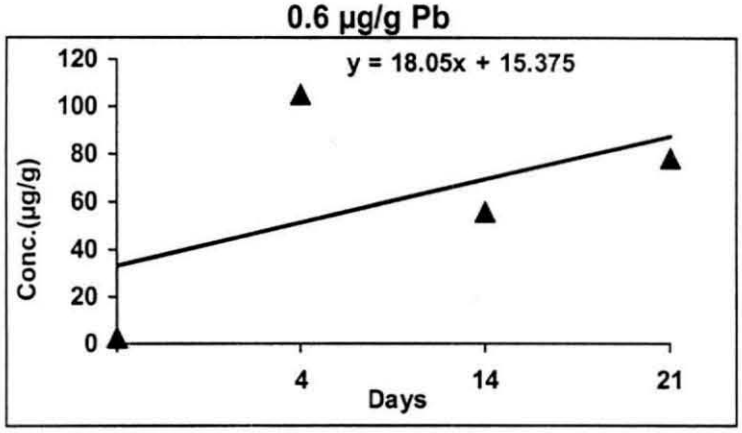
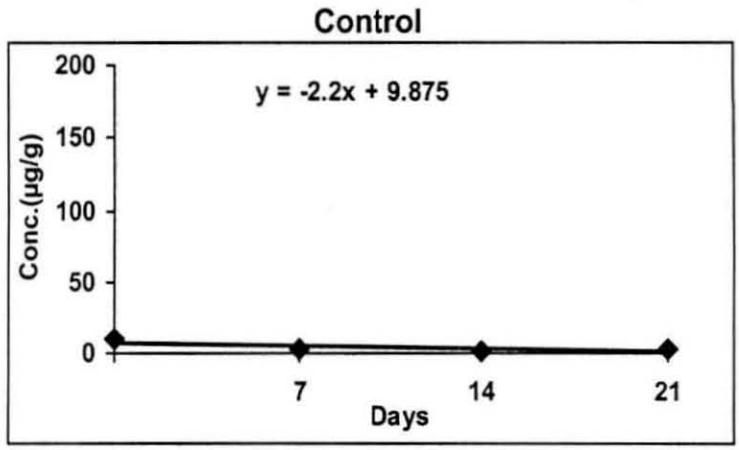
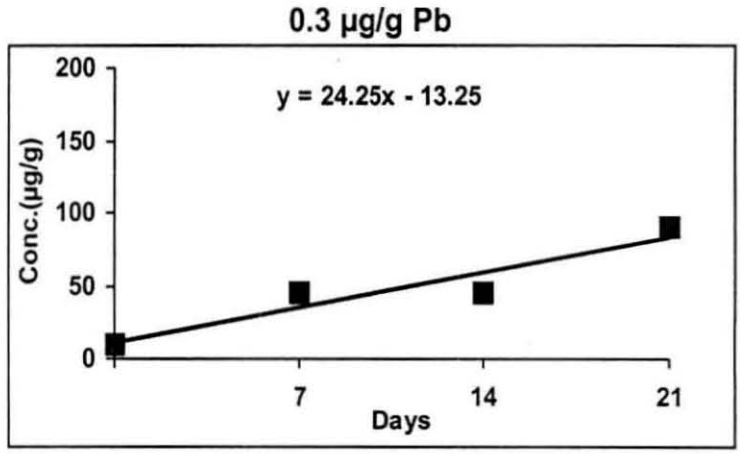


Fig. 28 : The linear relationship between time and concentration (lead) in the digestive glands of clams.





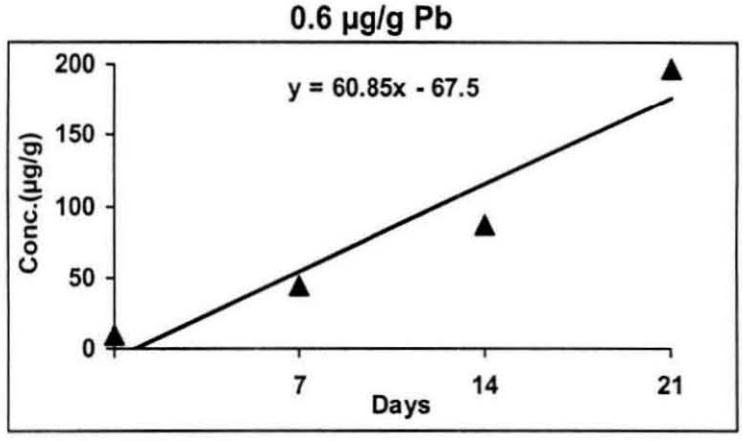
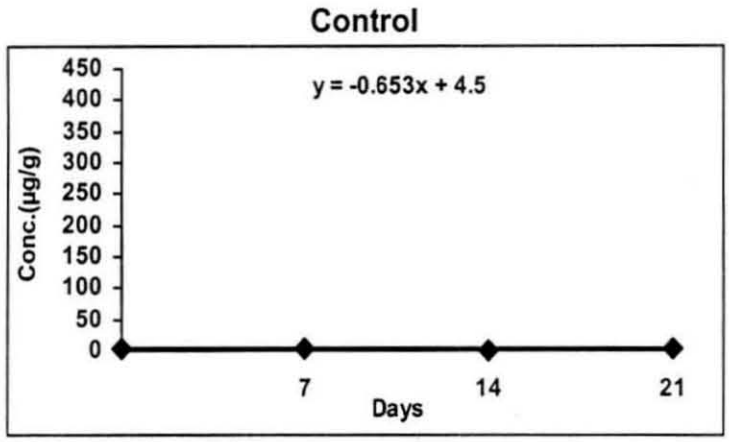
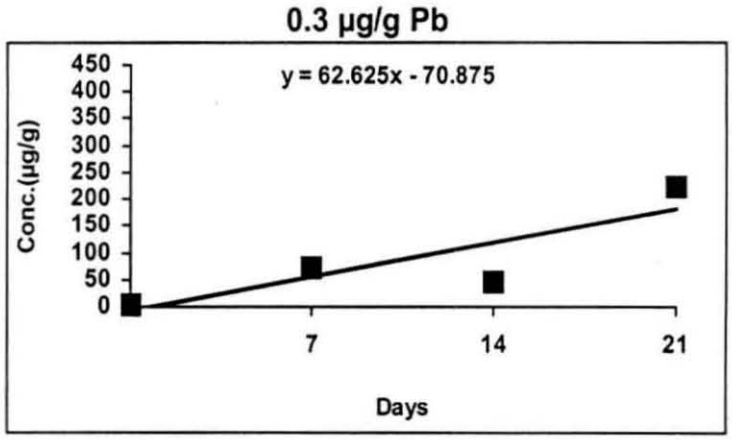
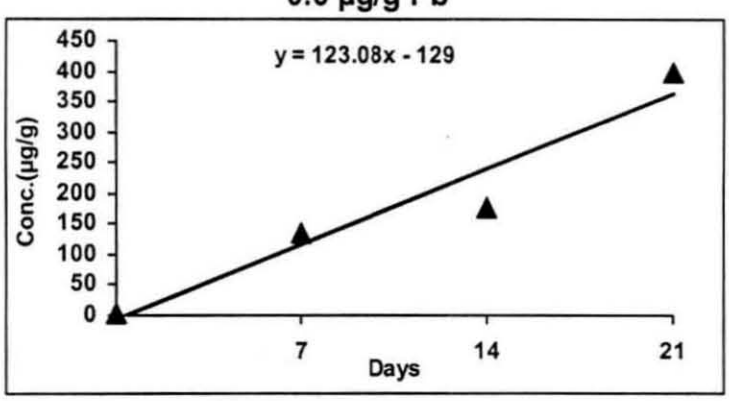


Fig. 29 : The linear relationship between time and concentration (lead) in the gills of clam.







was observed in the metal accumulation (see fig.27, 28 & 29). However, maximum accumulation was seen in gills with the accumulation in the digestive glands being slightly lower. The metal accumulation in whole soft tissue was considerably low.

Another interesting feature that was observed was that the increase in metal accumulation followed a linear relationship with time (see fig.24 to 29).

4.5. HISTOLOGY

Both qualitative and quantitative alterations which occurred in the cellular structure of clams exposed to copper (0.25 ppm) and lead (0.6 ppm) for 21 days were studied. In fact, both these alterations are interconnected. The following section deals with the qualitative aspects of the changes in the digestive tubules and gills.

4.5.1. The digestive gland

The digestive gland of molluscs consists of blindly ending tubules which are linked with the stomach by a system of branched, partially ciliated ducts. Tubules are more or less circular in cross section, surrounded by a sheath of collagen fibres and an external system of smooth muscle fibres forming a mesh work. The tubule epithelium

contains two cell types :

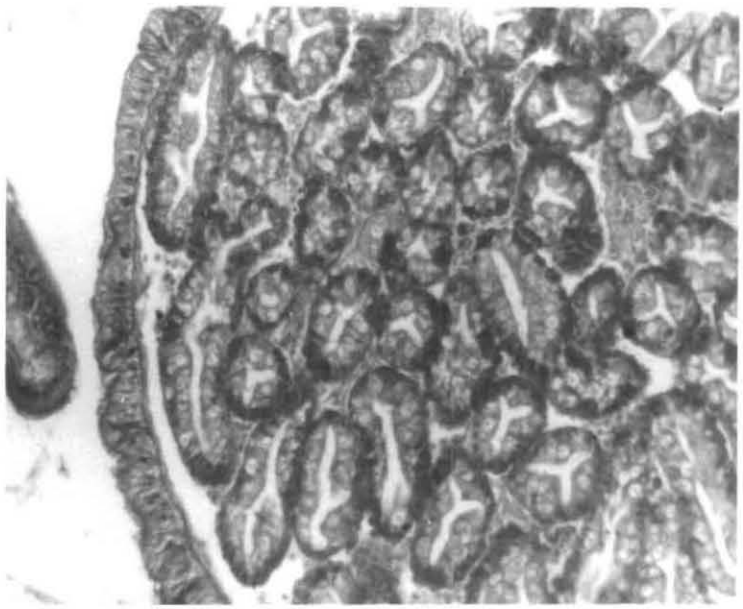
1) Acidophilic or digestive cells which are columnar and vacuolated. These cells are responsible for intracellular digestion of food.

2) Basophilic cells which are pyramidal, its function being secretory.

Photomicrograph 1 shows the digestive tubules of control animal.

4.5.2. Gills

The gills consist of four pairs of demibranchs which separate the pallial cavity into inhalent and exhalent chambers throughout its length. Each demibranch comprises two lamellae, one ascending and the other descending. The two lamellae are connected together by interlamellar junctions. Individual lamellae are formed of rows of ciliated filaments, which are joined to each other by ciliary interfilamentar junctions. A branchial vein runs through the length of the filament, its epithelium being formed of several ciliated or non- ciliated cell types. Innermost in the branchial vein are flattened epithelial cells that lie beneath the chitinous rod. The two rows of muscles viz., the frontal and the abfrontal, maintain the structural intergrity of the gill. The frontal portion of the filament is composed of columnar frontal cells with frontal cilia. Adjacent to these cells lie the large



Photomicrograph 1

Photomicrograph 2

Photomicrograph 1& 2 : C.S of digestive tubules and gill filaments of *V.cypnioides* maintained under control conditions x 20 x

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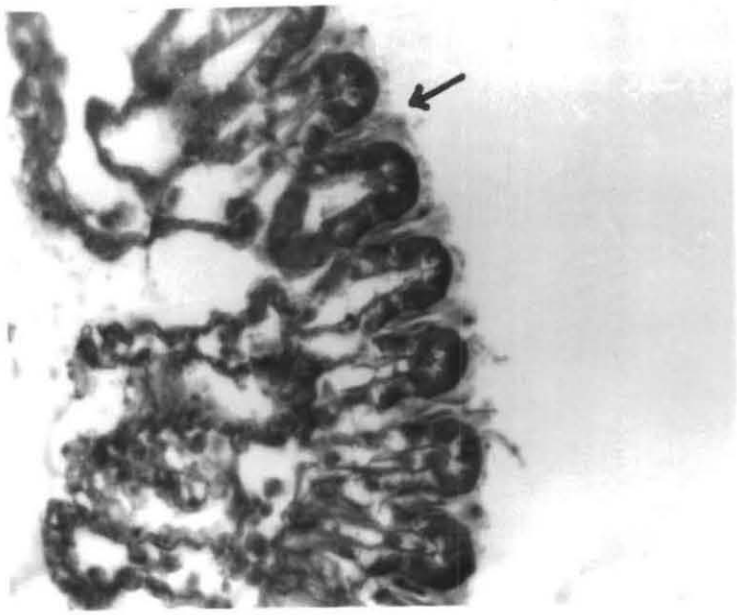
ciliated latero frontal cells. The post latero frontal cells are non-ciliated. A row of lateral cells bear the lateral cilia. Small, non- ciliated post lateral cells are gradually replaced by the squamous endothelial layer of the branchial vein. At the interfilamentar junction the endothelial cells are replaced by ciliary discs. The abfrontal end of the filament consists of abfrontal cells that asymmetrically bear the abfrontal cilia. Beneath the ciliated abfrontal cells are seen the mucus glands. Photomicrograph 2 and 3 shows the gills of the control animal. Frontal cilia, lateral cilia and interfilamentar junctions could be clearly seen.

4.5.3. Histopathology of the digestive gland

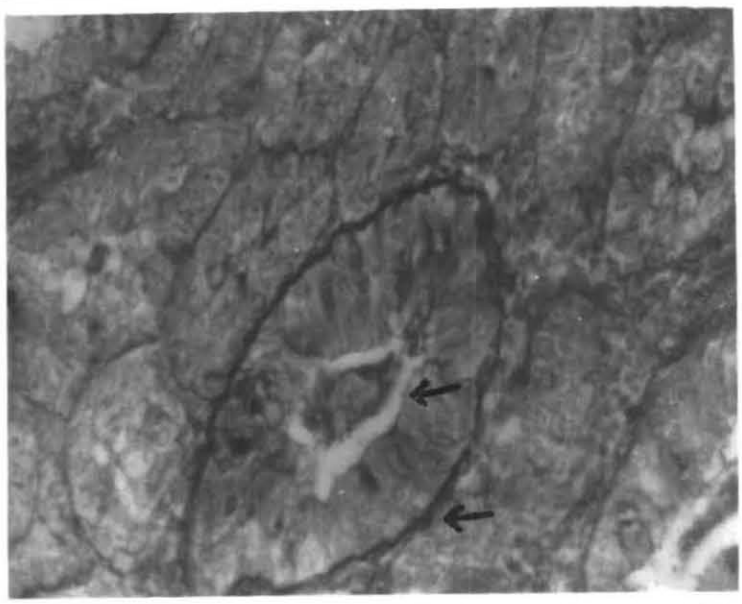
The pathological changes observed in the digestive gland of copper and lead treated clams were similar. However, some differences could be pointed out. The epithelial cells of the digestive tubule of copper treated clams showed considerable damage (Photomicrograph 4). The tubule lumen was obliterated and contained damaged cells that were sloughed off. Basal lamina get thickened. Cells lost integrity and remained indistinguishable. Complete destruction of digestive cells leading to vacoulation and even the disappearance of outer lamina was observed (Photomicrograph 5). Massive infiltration of haemocytes due to rupture of blood vessels were observed. Sometimes they aggregate to form clusters-granulocytoma (Photomicrograph 6).

Another observation was the enlargement of the tubules



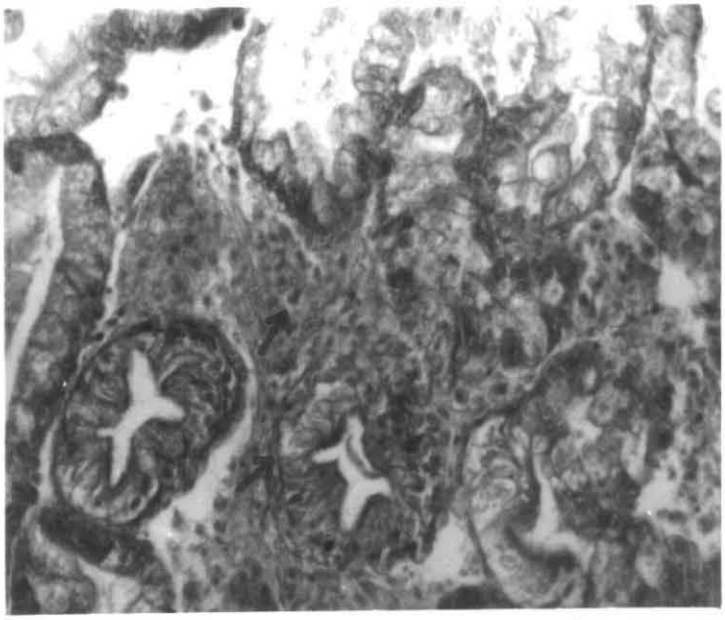
Photomicrograph 3

C. S. of gill filaments of *V. cyprinoides* maintained under control conditions. Arrow indicating interfilamentar juntion x 40 x



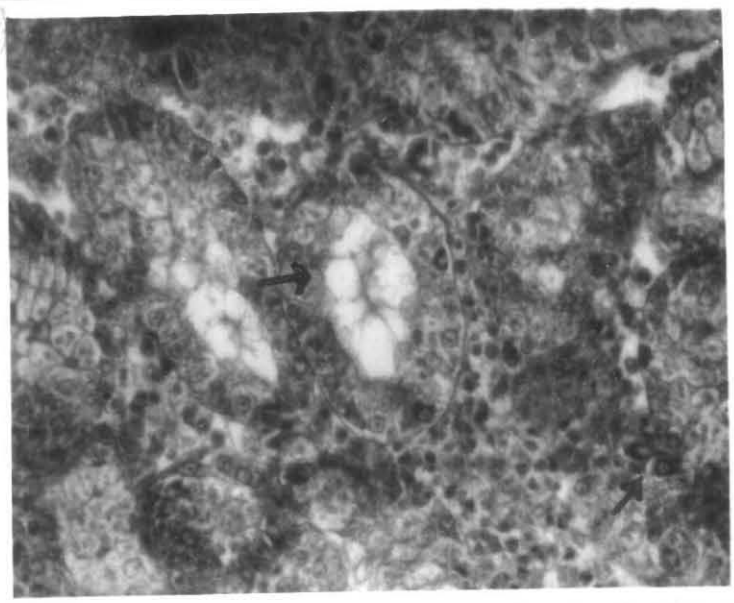
Photomicrograph 4

The digestive gland of *V. cyprinoides* exposed to copper (0.25ppm). Arrows indicating obliterated lumen and thickened basal lamina x 40 x



Photomicrograph 5

The digestive gland of *V. cyprinoides* exposed to copper (0.25ppm). Arrows indicate haemocyte infiltration and total destruction of digestive cell x 20 x

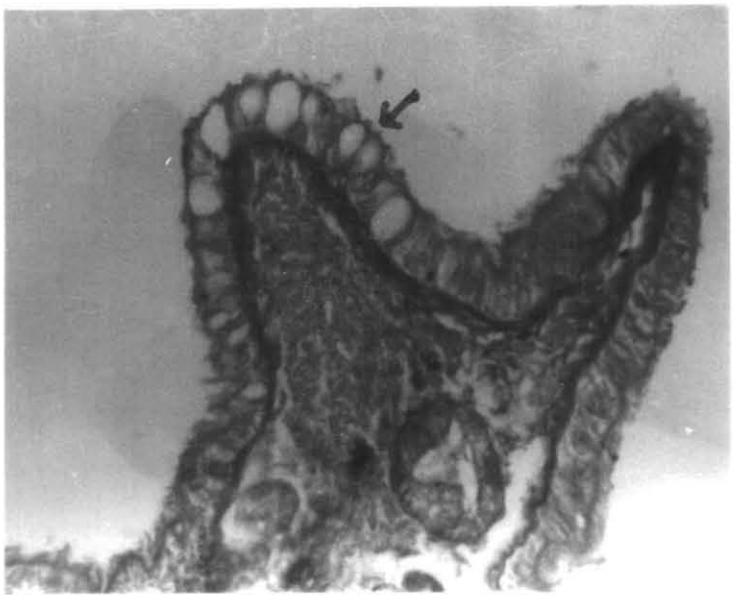


Photomicrograph 6

Arrows indicating granulocytoma, destruction of digestive tissue and obliteration of lumen in the digestive gland of *V. cyprinoides* exposed to copper (0.25ppm) x 20 x

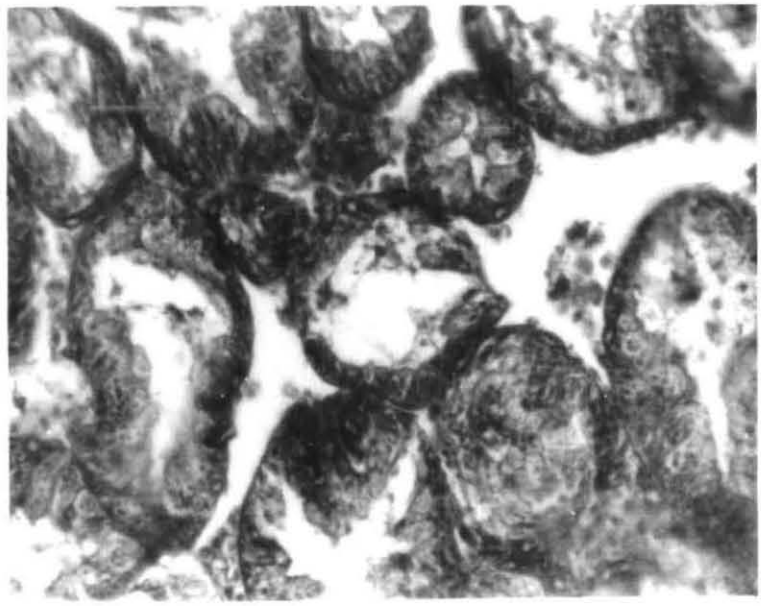
Photomicrograph 7

Arrow indicate loss of cilia in the digestive diverticula of *V. cyprinoides* exposed to copper x 20 x



Photomicrograph 8

Arrow indicate loss of cilia, bulging and vacoulation of columnar epithelial cells of digestive diverticula of *V. cyprinoides* exposed to lead (0.6ppm) x 40 x

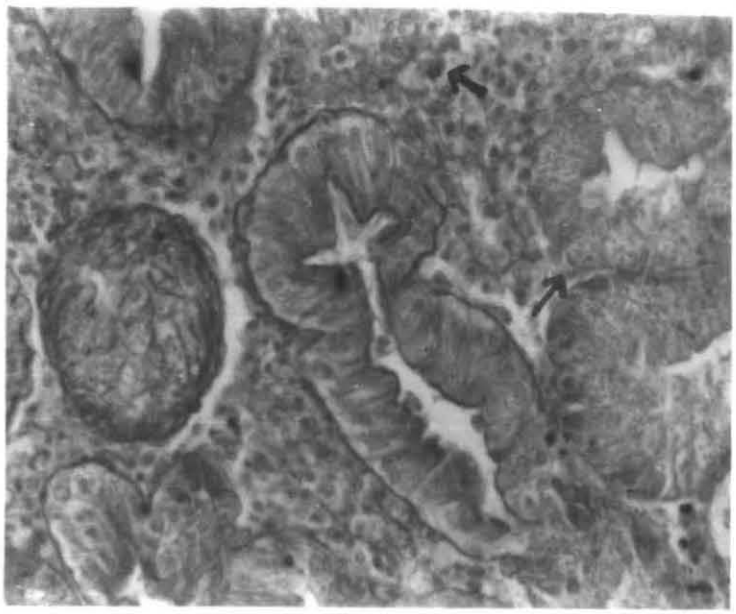


Photomicrograph 9

Digestive tubules of *V. cyprinoides* at station 5 during Post-monsoon season. Digestive tubules lost its integrity and cells become indistinguishable. Loss of tubules were also seen. x 40 x

Photomicrograph 10

Digestive tubules of *V. cyprinoides* at station 5 during Post-monsoon season. Tubules were dialated and severe destruction of digestive cells were seen. x 40 x



Photomicrograph 11

Digestive tubules of *V. cyprinoides* at station 2 during Post-monsoon season. Arrows indicate haemocyte infiltration, destruction of epithelial cells and loss of tubules. x 40 x

(Photomicrograph 7) and loss of cilia in the epithelium of the diverticula. There was reduction in epithelial thickness of the tubule. This may be due to the destruction of digestive cells and its replacement by the comparatively short basophilic cells.

An important pathological change observed in the digestive gland of lead treated clams was the bulging and vacoulation of the columnar epithelial cells of the digestive diverticula. Loss of cilia also could be seen (Photomicrograph 8). Destruction of digestive cells, increase of intertubular spaces and dialation of tubules were also observed.

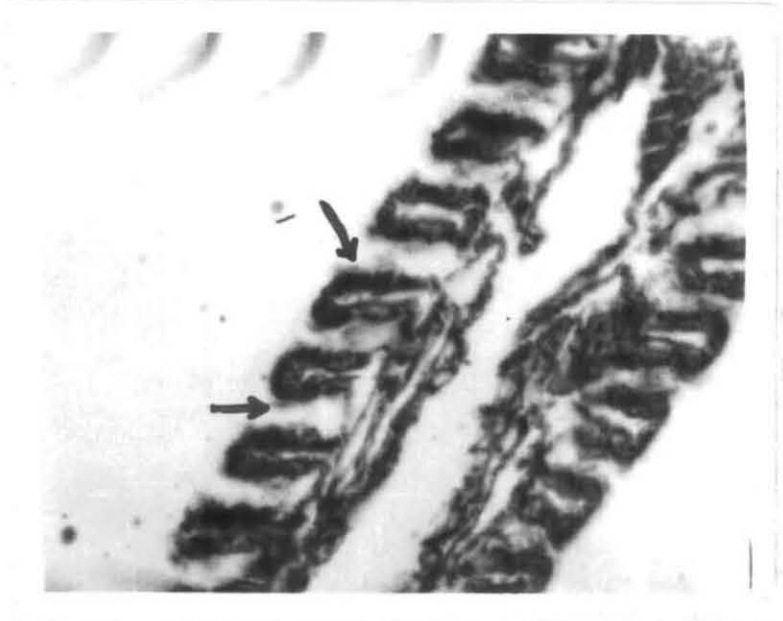
Histology of the digestive gland of *V. Cyprinoides* collected from station 5 & 2 showed considerable damage of the tissue. Extensive damage, dialation of the tubules, leading to their total destruction was observed. Haemocyte infiltration was another striking observation. The samples were collected during the post-monsoon season, When the mean copper concentration was high in the digestive glands. This heavy load of copper might be a factor contributing such a devastating effect on the tissue.

4.5.4. Histopathology of the gills

After 21 days exposure to copper, the gill filaments shrunk. There was loss of frontal cilia indicating damage to the frontal cells. Though the interfilamentar junctions were not much distorted, abfrontal cells

Photomicrograph 12

Arrows showing breakage at the interfilamentar junction and shrinkage of lamellae in the gills of *V. cyprinoides* exposed to copper (0.25ppm) x 40 x



Photomicrograph 13

The C. S of the gill of *V. cyprinoides* exposed to lead (0.6ppm). Arrows indicate shortening and flattenning of lamellae and total destruction of interfilamentar junctions x 40 x

lost cilia and got shrunk (Photomicrograph 12).

The gills of clams exposed to lead showed total destruction resulting in the absence of all the cilia. The gill lamellae were reduced in size and density (total number occupying a unit area). They became very short and flattened. Frontal cilia were lost completely. This may be due to the destruction of frontal cells. The interfilamentar junctions were found to be completely destroyed. In place of these, haemocyte granules were seen. Branchial vein was dialated. The chitinous rod was broken at several places due to damage of abfrontal and frontal muscles. The lamellae were detached at some places (Photomicrograph 13). The epithelium broke up and the inner parts of the filaments disappeared completely; the outer parts of the filaments became flattened.

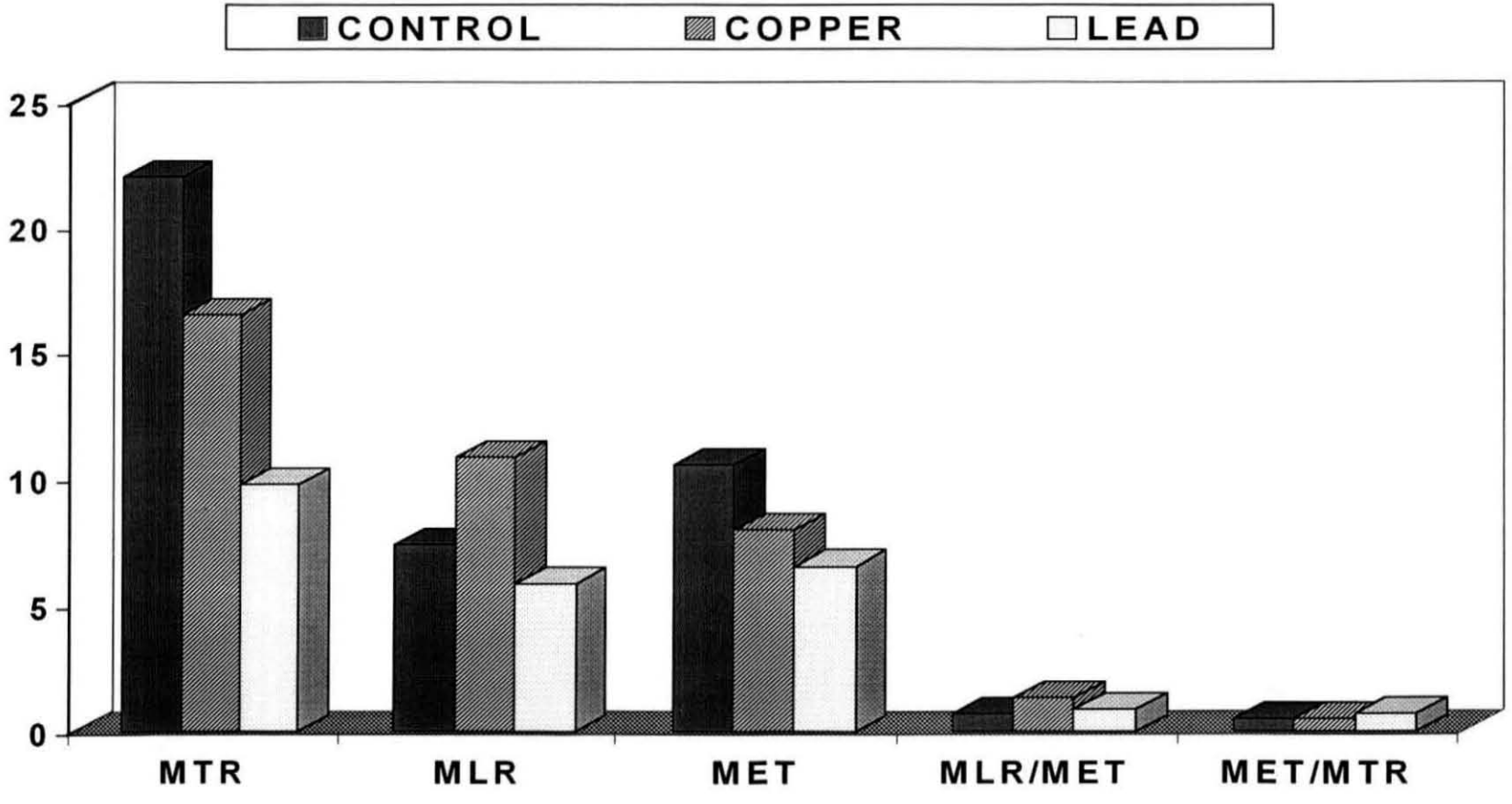
Copious secretion of mucus was observed in the gills of both copper and lead treated clams. This could be due to the metal ions that caused enlargement of mucus cells and this excess mucus production may be a defence mechanism. But the continous secretion of mucus, later leads to the exhaustion and finally destruction of these cells.

Both copper and lead caused severe damage to both the organs. However, it is to be noted that copper causes severe damage even at relatively low concentration. Hence copper is more toxic than lead for the organism.

4.6. PLANIMETRY

Bivalves have the ability to survive within the polluted environments in spite of the fact that the pollutant levels overload the normal limits. This results in the alterations of the normal cell structure, which will be more manifested in the digestive glands of these animals. These alterations can be regarded as cellular markers for studying the pollutant level in the environment as well as its effect on the organism. These alterations could be effectively studied using planimetric techniques. The study of the quantitative structure of the digestive tubules of clams exposed to metals (copper and lead) revealed that those clams exposed to copper (0.25 ppm) have low MET and MET/MTR ratio (see fig.30) which indicates poor cell condition. Low MET value indicate a reduction in the epithelial thickness of digestive cells of treated animals. Statistical tests (Fisher's Protected LSD, Scheffes'S & Dunnet one- tailed) showed significant differences in MET, MLR, MLR/MET & MET/MTR values of digestive tubules of copper exposed animals when compared to control. Though MET value declined for lead treated (0.6 ppm) animals, MTR value did not show any significant increase. The MLR, MET/MTR & MLR/MET values of lead treated animals did not show statistically any significance except for the MET value. Anova (Fisher's Protected LSD, Schiffes'S & Dunnet one-tailed) tests, showed significant differences for the planimetric parameters of copper exposed clams, which clearly shows the heavy toxicity of copper to the animals.

Fig: 30. The differences in MTR (Mean Tubular Radius), MLR (Mean Luminal Radius), MET (Mean Epithelial Thickness), MLR/MET & MET/MLR values of copper and lead exposed clams and the control ones.



4.7. ULTRASTRUCTURE

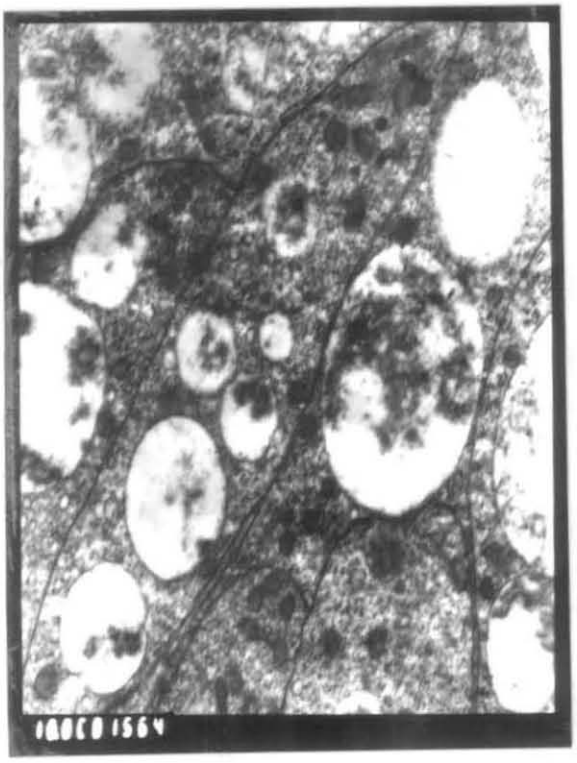
The fine structure of the digestive gland of *Villorita cyprinoides* exposed to copper (0.25 ppm) and lead (0.6 ppm) for 21 days was studied in detail. The normal epithelium of the digestive tubules consist of two cell types, viz, digestive or acidophilic cells and basophilic cells (see section 4.5.1.). The fine structure of the digestive cell is characterized by the presence of numerous cytoplasmic vesicles and by microvilli which project from the cell apex into the tubule lumen. Particulate material from the lumen is taken up at the base of the microvilli by pinocytosis and the pinocytotic vacoules so formed fuse giving rise to heterophagosomes. The digestive cell also contains mitochondria, golgi elements, free ribosomes and elements of both smooth and granular endoplasmic reticulam. The basophilic cells are of two types-mature secretory cells and immature flagellated cells.

The basophil cells are more or less pyramidal in shape (Owen, 1970) with the broad base resting on the basement membrane and the tapering apical portion with microvilli bordering the lumen of the tubules. The dominant feature is the presence of parallel arrays of granular endoplasmic reticulam in the basal, lateral and circum nuclear regions of the basophil cells. Other features include numerous free ribosomes, active golgi apparatus arranged more or less concentrically to enclose an extensive cup shaped golgi zone, mitochondria, membrane bound micro vesicles and secretory vesicles towards the cell apex. Basophil cells are sites of extensive protein synthesis. The immature flagellated basophil cells

differ from the secretory mature basophil cells in the presence of flagellum which project from the apical region of the cell into the lumen of the tubule. Other major differences are the scarcity of endoplasmic reticulam, presence of numerous free ribsomes distributed throughout the cytoplasm, golgi apparatus with only one or two stacks of saccules although not as prominent as that of a mature cell, presence of autophagic vacuoles etc.

Photomicrographs 14 & 15 shows a typical digestive cell of control *V. cyprinoides*. Lysosomes at various stages of digestion could be seen. Fusion of vesicles with contents to primary lysosomes for digestion (enzymatic) could be seen (Photomicrograph 15). This shows the process of digestion is taking place at a normal pace.

Under electronmicroscopy, copper exposed clams showed degeneration of cells. Nucleus was shrunken. Nucleolus and nuclear membrane were absent. The denuded cells indicates necrosis (Photomicrograph 16). The lysosomal number increased. Lysosomes appear large indicating fusion has taken place. A number of compressed mitochondria undergoing morphological changes were seen near the vacoulated digestive cells (Photomicrograph 17). Rupture in the basal lamina was observed with nucleus showing signs of degeneration with breakage of nuclear membrane. Nucleolus was absent. Loss of cytoplasm was also evident (Photomicrograph 18). Photomicrograph 19 shows a totally disintegrated nucleus pushed to the periphery of the cell with breakage of the nuclear membrane. Nucleolus was absent. Nuclear material was disintegrated and the nucleus was almost vacuolated. The cell lost most of its cytoplasmic



Photomicrograph 14
(x 10,000 x)

Photomicrograph 15
(x 8,000 x)

Photomicrographs 14 & 15 : Fine structure of digestive cells of *V. cyprinoides* maintained under control conditions .

organelles. All these observations leads one to infer that the cell was at the verge of destruction. There was an increase in the number of basophilic cells. Similarly there was extensive proliferation of lysosomes leading to the formation of primary, secondary and tertiary lysosomes. Breakage in the lysosomal membrane was also noticed.

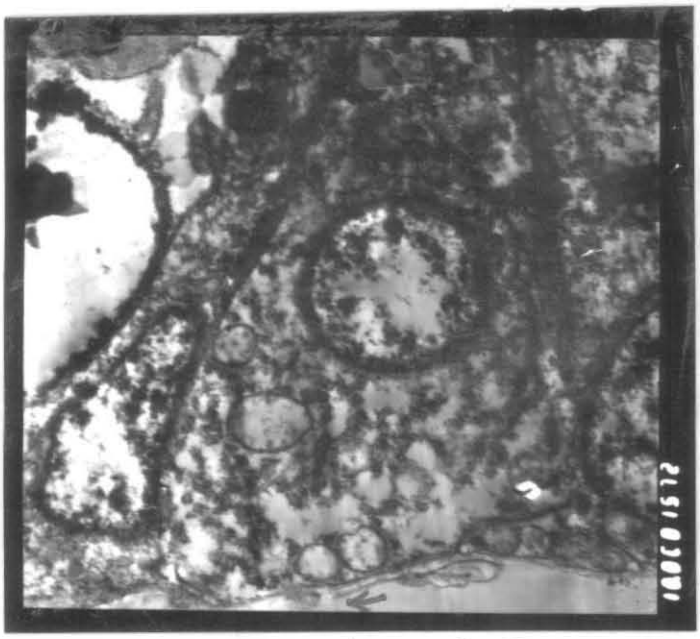
Photomicrograph 20 shows a basophilic cell undergoing destruction due to lead exposure. The cell lost its nucleus and a number of lysosomes were observed which were later seem to fuse. This process finally leads to the formation of enlarged lysosomes (heterolysosomes) which were less in number. Cell membrane shows signs of disintegration. There was extensive proliferation of smooth endoplasmic reticulam. Mitochondria appear compressed and shows signs of disintegration. The cell lost its villi and appeared bulged. A shrunken nucleus with labile nuclear membrane was seen, but the nucleolus remains intact. Photomicrograph 21 shows extensive proliferation of smooth endoplasmic reticulam, the number of mitochondria increased and showed morphological changes. Observation of digestive gland tissue of lead exposed clams also showed many changes in the nucleus. The nucleus was shrunken with a labile nuclear membrane, but the nucleolus remains unaffected. An extensive proliferation of smooth endoplasmic reticulam and an increase in the number of disintegrating mitochondria were indications of cellular pathology (Photomicrograph 21). In most cases, nucleus was severely affected. The enlarged lysosomes were often associated with disintegrating mitochondria. Reduction in lipid droplets were also noticed. Cytoplasmic organelles fuse with lysosomes which was manifested by the development of multilayered membrane to the lysosome (Photomicrographs 22 & 23). The number of golgi bodies were also found to be increased.

The fine structure of digestive tubule of *V. cyprinoides* exposed to copper (0.25ppm). Arrows indicate loss of cytoplasm and degeneration of the nuclear material x 50,000 x

Photomicrograph 16

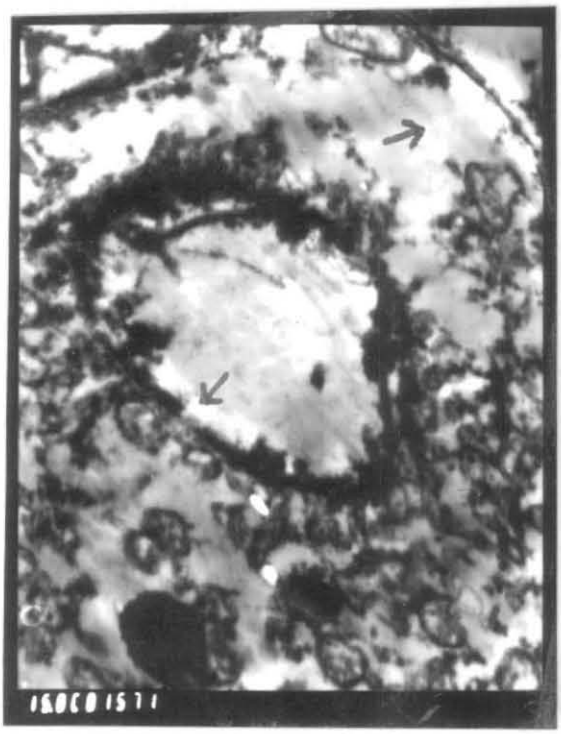
The fine structure of digestive tubule of the clam exposed to copper (0.25ppm). Arrow indicate compressed mitochondria undergoing morphological changes x 10,000 x

Photomicrograph 17



Arrow indicating breakage at the basal lamina of the basophilic cell of *V.cyprinoides* exposed to copper (0.25ppm) x 10,000 x

Photomicrograph 18

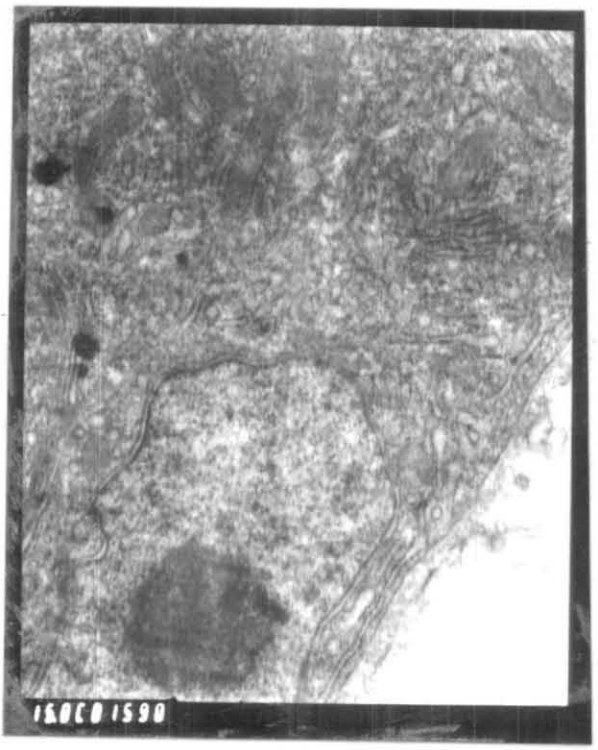


Arrows indicating loss of cytoplasm and labile nuclear membrane of the digestive gland cell of *V.cyprinoides* exposed to copper (0.25ppm) x 15,000 x

Photomicrograph 19

Proliferation and fusion of primary and secondary lysosomes which later forms larger tertiary lysosomes in the digestive gland of *V.cyprinoides* exposed to lead (0.6ppm) x 10,000 x

Photomicrograph 20



The fine structure of the digestive gland cell of *V.cyprinoides* exposed to lead (0.6ppm). Extensive proliferation of SER, RER and golgi bodies could be seen. Nucleus is highly active, synthesising protein - may be an immune response x 5,000 x

Photomicrograph 21

Fine structure of the digestive gland cell of *V.cyprinoides* exposed to lead (0.6ppm). Disintegration of mitochondria and fusion of lysosomes could be seen x 10,000 x

Photomicrograph 22

Arrows indicate fusion of cell organelles with lysosome which was evidenced by the formation of multilayered membrane of the latter x 10,000 x

Photomicrograph 23

5. DISCUSSION

5.1. FIELD-STUDY

The trace metal concentrations in coastal waters depend on the intermixing, initial concentrations of metals and physico-chemical conditions in the mixing zone. These changes can lead to precipitation and adsorption. It appears that the actual concentrations of heavy metals are many orders of magnitude lower than that predicted by thermodynamic considerations. Thus for a proper understanding, it is necessary to analyse the bottom deposits for trace metals (Iyer, 1994).

Of the four stations selected for the regular monitoring in the present study, station 5(Chembu) recorded higher level for copper in the sediment during post-monsoon months and the minimum level during pre-monsoon months. For the other three stations values were relatively low and significant seasonal pattern could not be observed. For lead, station5 showed high values during all the months with highest level in the post-monsoon months. Other three stations did not show any seasonal trend in metal concentrations. For cadmium, seasonal variation could not be observed in any of the stations.

Venugopal et al., (1982) reported a high value (70.8 ppm) of copper from Cochin backwaters. In the above study, significant increase in

copper was noted during the monsoon seasons. The authors attributed this enrichment to the enhanced inflow during monsoon and subsequent mixing and settling of the metal in the water column and sediment. The settling of the trace metals in the estuaries was also attributed to flocculation and sedimentation by Dyer (1972).

High values for copper from Cochin backwaters were reported by Nair et al., (1990); Jayasree & Nair, (1995) reported low values of copper in ,the Cochin estuary. They obtained comparatively low values towards the marine side of the estuary and the high values to the fresh water ends. They attributed the high values to settling of the metal by flocculation and sedimentation process due to change in salinity. In the present study, station 5, where the salinity was very low showed high values for copper which could be due to the settling of the metal in the area.

Rajathy & Paul Azariah (1996) reported elevated levels of copper in the industrial region of Ennore estuary during monsoon and post-monsoon seasons whereas low values were recorded during the summer months. The results of the present study are in conformity with the above findings.

Earlier reports of high incidence of cadmium in the Cochin back waters was explained as a result of anthropogenic sources mainly industrial and domestic effluents (Ramalingam Pillai et al., 1994). The same author reported high values for lead (38.48 ppm) from the same area.

A seasonal distribution of lead in Cochin estuary was reported by Nair et al.,(1990). They recorded high values for lead during pre-monsoon and post-monsoon periods but low values during monsoon. Jayasree & Nair (1995) reported low concentrations of lead towards the fresh water zone of the Cochin estuary and they attribute this observation to the sandy nature of the sediment as was proposed earlier by Zindge et al., (1998).

In the present study, comparatively high values were obtained for copper in the whole soft tissue of clams in all the stations. Maximum values for copper and cadmium were observed during monsoon and post-monsoon seasons coinciding with a low saline condition. For lead, a seasonal variation could not be observed. Senthilnathan & Balasubramaniam (1998) showed significant seasonal pattern of metal (zinc, cadmium & lead) distribution in the tissues of the oyster *Crassostrea madrasensis* from the estuaries of south east coast of India. They observed high level of metals during the monsoon season and low levels in the summer months except for copper. They attributed this high level of metal concentrations during the monsoon months to the influx of metal rich fresh water carrying agricultural and industrial wastes into the estuaries.

Senthilnathan et al., (1998) obtained a clear significant seasonal pattern of metal load (lead, zinc, copper and cadmium) in the tissues of mussel *Perna viridis* and oyster *Crassostrea madrasensis* with high level during the monsoon season. Similar observations were reported in *Mytilus edulis* and oyster from Narraganser Bay (Farrington et al., 1983) from

Bay of Bourgmeuf (Amiard et al., 1986) in oyster (Rajendran et al., 1988) in mussels (Rivoniker & Parulekar, 1998) in the bivalve *Sunetta scripta* (Pillai & Valsala, 1995). All the above results were explained on the basis that during monsoon season, the added influx of river water with suspended sediment particles into the system contribute considerably to the metal load (Senthilnathan & Balasubramaniam, 1998; Pillai & Valsala, 1995). Comparatively high values obtained for copper, in the present study also could be due to the above reason.

The metal levels in molluscs are usually linked to size, sex, reproductive condition, seasonal variation and also on the available chemical form of metals in the ambient water which in turn facilitates the intake by the organism (Pillai & Valsala, 1995; Phillips & Rainbow, 1997). Trace metals dissolved in the sea are partitioned in equilibria between complexing ligands, inorganic and occasionally organic form. Numerous processes influence the concentration and bioavailability of a metal in the marine environment (Bryan & Langston, 1992). These processes are, inturn affected by environmental factors such as salinity, the redox potential of the sediment, temperature and PH.

Copper is complexed by inorganic chelating agents such as carbonate and hydroxide (Bruland, 1983). The free metal ion released from these chemical equilibria in solution would be the form available for binding to a membrane transport ligand and therefore represents the bioavailable form of the metal (Williams, 1981a; b; Phillips & Rainbow, 1993). The ionic form of copper is the most toxic (Salomons & Forstner,

1984; Langston, 1990; Meador, 1991; Meador et al., 1993). The effect of salinity on the free ionic activity of the metal was given by Zamuda & Sunda, (1982). The increased copper uptake in oysters (*C.virginica*) under low saline conditions could well explained in the light of the above mentioned work. Similarly in the present work, increased accumulation of copper found in the whole soft tissue and digestive gland of clams under low saline conditions (during monsoon and post-monsoon) could also be substantiated on the basis of the previous work studies.

Cadmium in sea water exists mainly as chloro-complexes (Cd cl^{+}, Cd cl_2, Cd cl_3) with only 2.5 % present as free (hydrated) ions Cd^{2+} (Zirino & Yamamoto, 1972; Bruland, 1983; Rainbow et al., 1993). Phillips & Rainbow (1989) stressed the importance of water quality parameters like temperature and salinity in influencing metal (pollutant) level in the organism. The effect of salinity of the ambient medium on the accumulation of metals in the tissue is further substantiated by several authors (Phillips, 1979; Luoma & Cain, 1979; Peerzada & Dickinson, 1989; Pillai & Valsala, 1995). Reduction in salinity necessarily decreases the chloride concentration of the ambient medium with automatic reduction of the chlorocomplexation of cadmium ions (Bruland, 1983) and corresponding increases in the availabilities in the free metal ions.

Shibu et al., (1990) found that copper in the waters of Cochin estuary was extensively associated with organic colloidal matter (as well as with particles). Moreover, a high proportion of total copper has been shown to be associated with organic species. The significance of the above

observation was more while considering the findings of Viarengo et al., (1993) who reported that the accumulation of copper in the digestive gland of mussels increased when the metal is associated with particulate matter. The increase in concentration of copper recorded in the present study, in the digestive gland of clams can be well explained on the light of the above findings.

In the present study, lead accumulation in the digestive gland did not show any specific pattern. Though maximum value was reported at station 5 in the pre-monsoon months when the salinity was slightly high but the salinity of the station was low compared to the other three stations. This low salinity may be a precipitating factor in the increased load of the metal in the digestive gland. Regarding the influence of salinity on the uptake of cadmium and lead, many studies had been conducted earlier. Phillips (1976) found that for mussels (*M.edulis*), cadmium uptake increased at low salinities whilst lead uptake decreased at low salinities. However, this view contradicts with the results of the present study.

5.2. Metal accumulation dependence on clam size

In the present study, larger size groups (30-40mm) from station 1 and 5 (except station5 for lead) showed slightly high values for the metals (copper, cadmium and lead) than the small size groups i.e. an increase in the accumulation of metals was observed with increasing size.

The influence of animal size (not always equated with age) on metal accumulation was given by several workers (Boyden, 1974; 1977; Phillips, 1976a; Manly & George, 1977; Davies & Pirrie, 1978; Harris et al., 1979; Cooper et al., 1982; Popham & D' Auria, 1983; Simpson, 1979; Cossa et al., 1980; Boalch et al., 1981). Romeril (1971) reported an increase in accumulation of metal with increasing size for copper in the clam *Mercenaria mercenaria* but later he (1974) found that copper concentrations in the same species decreased with age. Ayling (1974) reported an increase in the concentration of cadmium, copper and lead with an increase in age in Pacific *oyster C.gigas*. The increase in concentration of metals with increase in age was explained by Jones & Walker (1979). They stated that metals are accumulated in excess of metabolic needs and excretory capacity and that the excess is stored. As the animal gets older, the amount of stored metal increases more rapidly than body weight.

Watling & Watling (1976) reported higher levels of copper in young mussels. Williamson(1980) reported high concentration of zinc accumulated in small size groups compared to larger ones in a population of snails. He correlated the findings with the metabolic activity of the animal. He suggested that the increase in metabolic rates in younger individuals may affect metal uptake and elimination differentially. Claude & Maureeni (1999) stated a size dependent difference in metal accumulation. According to them, small cockles accumulated a higher level of metals than larger ones and it was mentioned as a 'dilution effect'.

5.3. ACUTE TOXICITY

The study conducted on LC 50 indicated that copper was more toxic to the animal compared to lead. The heavy toxicity of copper to the organisms compared to other essential trace metals was earlier reported (Sosnowski & Gentile, 1978; Johnson & Gentile, 1979; Sunda et al., 1987; Paulson et al., 1989; Ahsanullah & Williams, 1991). For the same species varying LC50 values were reported earlier. Some of these reported values and the values obtained in the present study are given in table.5(I).

Chan, 1988 reported that *Perna viridis* was more sensitive to copper and less sensitive to lead. In the present study, *Villorita cyprinoides* was a more tolerant species compared to the mussels (as could be seen from the table). The animal is more sensitive to copper than to lead. Being a euryhaline species the animal can tolerate very low salinity. In the present study, the salinity which was used during the experiment was 10 ppt. This low salinity might be a contributory factor for the comparatively high value of LC 50 for copper and which makes the animal one of the best species to be used as the sentinel organism in the coastal areas.

5.4. BIO-ACCUMULATION

In the present study, after exposure (21 days) to different concentrations (0.1 & 0.25 ppm) of copper, digestive gland showed maximum metal accumulation followed by gills and whole soft tissue whereas

Table 5(i) : Some of the acute toxicity data published for Cu & Pb to bivalves

Metal	Species	LC_{50}(mg/L)	Salinity	Source
Copper	V. cyprinoides	2000(24h)	1	Lakshmanan & Nambisan(1977)
Copper	P. viridis	0.14(48h)	33 ±1	D'Silva & Kureishy(1978)
Copper	M. edulis	480(96h)	33 ±1	Amiard-Triquet et al.,(1986)
Copper	M. edulis	1550(96h)	33 ±1	Amiard-Triquet et al.,(1986)
Copper	P. viridis	0.174(96h)	25	Nambisan & Lakshmanan(1986)
Copper	P. viridis	0.086(96h)	35	Krishna Kumar, 1987b
Copper	V. cyprinoides	1179(96h)	10 ± 1	Present Study
Copper	P. viridis	620(96h)	32	Chan(1988)
Lead	P. viridis	4460(168h)	32	Tan & Lim(1984)
Lead	P. viridis	8820(96h)	32	Chan(1988)
Lead	V. cyprinoides	4000(96h)	10 ± 1	Present Study

exposure to different concentrations (0.3 & 0.6 ppm) of lead showed maximum accumulation in the gills followed by digestive gland. Earlier studies by Janssen & Scholz (1979) who exposed *Mytilus edulis* to cadmium for 21 days had shown high concentration in the digestive gland and followed by the gills, which is similar to the results reported in the present study for copper.

Gills were described as the largest organ of accumulation of copper where it may affect the ciliary feeding activity (Widdows & Donkin, 1991). Soto et al., (1997) reported raised copper levels in gills after exposure to the metal. Similar findings were also reported by several workers (Coombs, 1972; Viarengo et al., 1981; Soto et al., 1996a). High level of copper was reported in the gills of clam *Tridacna crocea* under natural and laboratory conditions (Duquesne & Coll, 1995). Pore cells which are found underneath the gill epithelium was found to be involved in haemocyanin metabolism (Mason & Nott, 1981; Mason, 1983). Haemocyanin is a blood pigment containing copper. For effecting the metabolism of this pigment copper is stored in the pore cells. This was reported to contribute to the raised metal level in the gills (Soto et al., 1996b).

Bivalves obtain metal through food and by direct uptake from the sea water. The intracellular digestion and absorption of the food takes place in the digestive cells of the digestive gland. This may explain the concentration of metals in the particular organ (Krishnakumar, 1990a). The differential accumulation of copper by the digestive gland and the gills was

given by Viarengo et al. (1993). He observed that copper was accumulated by the mussels mainly in the gills when the former was present as a solute in the sea water. However, when copper was present in the sea water associated with particulate matter, higher accumulation of copper was found in the digestive gland in comparison with the gills. Segar et al., (1971) reported high levels of cadmium accumulation in the gut of *Pecten maximus* and in the mantle and gills of *Modiolus modiolus*. Pedersen et al., (1997) in an exposure study reported elevated levels of copper in the midgut gland and gills of shore crab *(C.maenas)*. They reported that copper is stored in the midgut gland by a special storage mechanism like granule formation. In mussels, organ specific accumulation have been reported for cadmium and lead (Schulz-Baldes, 1974; Denton & Burdon Jones, 1981; Roesijadi et al., 1984; Regoli & Orlando, 1994a). Roesijadi et al., (1984) in a study, comparing soft tissue, gills and digestive gland reported that gills and digestive gland accumulate a steady concentration of metals compared to other soft tissues.

Coombs & George (1978) stated that lead was accumulated in membrane bound vessicles of gills, viscera and kidney of the blue mussels and the mechanism was described as a type of detoxication. This could be a possible explanation for the elevated levels of lead found in the gills of the clam in the present study. George et al., (1978) and Rajendran et al., (1988) reported heavy accumulation of metals particularly copper in the gills of bivalves. The explanation given for the heavy load of metal in the gills was that the organ which is responsible for the water flow through bivalves and filter feeding are continuously exposed to

metal accumulation than other body parts. Mucus layer covering the gill and mantle may also cause high metal accumulation which was reported in *C.madrasensis* (Kumaraguru & Ramamoorthy, 1978). The mucus layer facilitates rapid accumulation because of the ion exchange properties (Pringle et al., 1968) .

Pringle et al. (1968), suggested that metal uptake by mussels is directly proportional to the external concentration. In the present study, a linear relationship was observed between time and accumulation rate and also between time and external concentration of both the metals (copper and lead) in the whole soft tissue, gills and digestive gland of clams for various concentrations. Silva & Kureishy (1978) & Ritz et al. (1982) found a linear relationship between toxicity of copper and concentration. A similar relationship was obtained for lead (Schulz-Baldes, 1974; Tan & Lim, 1984) and for cadmium (Ritz et al., 1982; Amiardd-Triquet et al., 1986). Bryan (1980; 1984) proposed a linear (positive) relation between the concentration of the pollutant in the environment and the animal. A linear relationship between time and accumulation of cadmium was established in the clam *Ruditapes decussataa* (Bebianno et al., 1993). Senthilnathan & Balasubramaniam (1998) obtained a linear relationship in metal accumulated by whole body tissue and different organs of oyster *C.madrasensis* with dissolved metal concentrations in the ambient medium. This could be due to increased absorption of metal on the body surface (Martincic et al., 1986) and could also be due to the uptake of organic ligand complexed metals through soft surface part of bivalves (Jorgensen, 1983). Sydney rock oyster *Saccostrea commercialis* exposed to different concentrations of

cadmium showed high concentration of the metal in the gut, kidney and gill. Visceral mass showed highest concentration. In the above study, cadmium accumulation was found to be non-linear in some concentrations (Trevor, 1982). Marigomez et al. (1989), in an exposure study on the marine prosobranch *Littorina littorea* reported that cadmium accumulation by the whole soft body, digestive gland and gills was linear and the rates of accumulation increased significantly with increasing cadmium concentration in the medium.

5.5. HISTOLOGY

5.5.1. Digestive gland

The digestive tubules of molluscan digestive gland is composed of digestive cells and basophilic cells. As a result of stressors like metals, chemicals or other contaminants there occurs an increase in the number of basophilic cells which in turn results in a decrease in the number of digestive cells (Widdows et al., 1984; Lowe & Clark, 1989; Cajaraville et al., 1990a; b; Marigomez et al., 1990a).

Light microscopy of digestive tubules revealed an increase in the number of basophilic cells and a considerable reduction in the number of digestive cells of copper and lead exposed clams. This results in comparatively shortened cells which consequently results in the reduction of

epithelial thickness. The increase in the number of basophilic cells was associated with degenerative changes in digestive cells (Thompson et al., 1974; Rasmussen et al., 1983a;b; Querubin & Enriquez, 1989; Cajaraville et al., 1990a; Marigomez et al., 1990a).

The exposure to adverse conditions or pollutants hamper the normal intracellular digestion taking place in the digestive cells, which in turn ,may lead, in basophilic cells, to an enhanced secretion of proteins for extracellular digestion (Marigomez et al.,1990). This intense and continuous secretion of protein results in the exhaustion of the organelle. This could be manifested in the disorganization of endoplasmic reticulam (Janssen & Ertelt-Janssen, 1983; Papathanassiou & King, 1986; Langston & Zhou, 1986).

Thompson et al.(1974) suggested that replacement of digestive cells occurs by division of (a type of) basophilic cell. Thus proliferation of basophilic cells could be related to an increased cell turnover and regeneration in damaged digestive tubules (Cajaraville et al.,1990a).

The digestive gland tissue is known to be the major target site for metal accumulation and is considered as a primary accumulator organ (Hemelraad & Herwig, 1988). In marine molluscs, the basal lamina of digestive tubules was reported to accumulate metals (Janssen & Scholz, 1979; Calabrese et al., 1984; Marigomez et al., 1990a; Soto, 1995). But a contradictory result was obtained in *Mytilus galloprovincialis* exposed to copper and zinc (Soto et al., 1996). In the present study, the basal lamina

appeared thickened and this could be attributed to accumulation of the metal by that portion.

The degenerative changes observed in the digestive gland of copper and lead exposed clams include, tubule dialation, breakdown, obliteration of the lumen, sloughing off of digestive cells, loss of cilia in the digestive diverticula. Similar changes were reported earlier in *M.edulis* exposed to trace metals and hydrocarbons (Calabrese et al., 1984; Auffret, 1988; Lowe, 1988) and in the digestive gland of mussel *P.viridis* exposed to copper and mercury (Krishnakumar et al., 1990). These changes may be due to the autolytic processes as a consequence of lysosomal destabilization. Dysfunction of the lysosomal system would lead to increased autophagocytosis and increased vacuolar fusion (Auffret, 1988; Moore, 1988a). This would in turn lead to atrophy of the digestive cells resulting in the observed dialation and degeneration of the digestive tubules (Lowe et al., 1981; Calabrese et al., 1984; Lowe, 1988).

In the present study, massive infiltration of haemocytes was observed in the digestive gland of both copper and lead treated clams. The involvement of haemocytes and pore cells in the mobilization of metals under normal physiological condition is well known (Soto et al., 1996b; Mason & Simkiss, 1983; Farley, 1988; Marigomez et al., 1990a; b; Nott et al., 1993). The haemocytes were produced as an immune response, they are involved in cellular defence mechanisms and toxicant uptake (Marigomez et al., 1990b). Brown cells which are also phagocytic occur along with haemocytes or haemocytes serve as vehicles for the transport of brown cells

(Robinson & Ryan, 1988). Though haemocyte infiltration is a defence mechanism, their frequent and intense infiltration through the stomach epithelium or the gill can result in the breakdown of cells (Marigomez et al.,1990).

Granulocytomas found in the digestive gland of metal exposed clams were reported earlier in mussel *M.edulis* from polluted environments (Auffret,1988) and the same author explained the findings as an inflammatory response and a result of chronic pollutant exposure. In the above work, another finding was that mussels exposed to diesel oil and copper mixture under laboratory conditions did not develop granulocytoma. Rasmussen et al. (1983b) induced a similar pathology in mussels after chemical exposure.

5.5.2. Gills

Gills are the most suitable structures to study the acute histological responses of the tissues due to exposure to pollutants (Sunila, 1988a). This is due to the fact that the epithelium of the gills are composed of several different cell types (Sunila, 1986). Moreover, one of the main pathways of metal ion entry into bivalves is passive diffusion across the gill epithelium (George & Pirie, 1980; Scholz, 1980; Carpene & George, 1981; Viarengo et al., 1981; Hietanem et al., 1988; Viarengo, 1989; Everaarts, 1990). Frontal and abfrontal cells of gills were the severely affected cells of the gill epithelium due to copper exposure (Sunila, 1988).

In the present study also, similar observations were noticed. Soto et al., (1996a) in a copper exposure study in *M. galloprivincialis*, located BSD (Black Silver Deposits) in the cytoplasmic granules of frontal cells. These granules might be lysosomes involved in the exchange of excess metals as a detoxication mechanism (Everaarts, 1990; Marigomez et al., 1990a).

Detachment of lateral cells, dialation of branchial vein, sloughing off of endothelial cells and presence of granular haemocytes were observed in the present study and could be inferred as an inflammatory reaction (Sunila, 1988a;b).

Another interesting observation in the present study was the excess mucus production in the gills during the exposure study. This excess mucus production is a defence mechanism and it stimulates the concerned cells to over activity thus resulting in the enlargement of mucus cells. The continued and excess production of mucus results in lowering of efficiency of the mucus cells. The enhanced mucus secretion acts as a coupling protective barrier against metal entry (Coombs, 1972), or as a detoxication mechanism to excrete excess metals bound to mucus components (George & Pirie, 1980). The fact that enlargement of gill cells is often accompanied by the presence of haemocytes, indicates tissue damage as well, mainly in the form of inflammation. As a result the gills become less efficient. The enlargement of gill cells and other related changes were reported in *M. edulis* exposed to cadmium (Sunila,1986), in *Anabas testudineus* after exposure to crude oil (Prasad, 1991) and in *Perna viridis* exposed to petroleum hydrocarbons (Nandini Menon, 1997).

5.6. PLANIMETRY

In the present study, MLR, MET, MTR and MLR/MET values for copper exposed animals showed significant variations from that of control animals. For animals exposed to lead, only MET showed significant deviation while, MLR, MTR and MLR/MET and MET/MTR did not show any significant deviation from the control values.

Two types of digestive activity have been distinguished in molluscs : monophasic and diphasic digestion. In monophasic digestion, there is always a predominant tubule type which is dependent on the specific phase of digestion in which the digestive gland is involved and which is related to food availability (Marigomez et al., 1990d). Morphologically, there are five types of tubules in the digestive gland of molluscs (Cajaraville et al., 1992).

i. Holding (H)

ii. Absorptive(A)

iii. Disintegrating(D)

iv. Reconstituting(R)

v. Necrotic(N)

The most abundant and common ones are H and A types in continuous and diphasic digestion. High percentages of absorpting digestive tubules seem to reflect a good nutritional or general condition, while increased numbers of reconstituting (R) appear to be indicative of malnutrition or of general poor condition (Cajaraville et al., 1992). In diphasic digestion, substitution of H type of tubules by digestive (A) tubules causing an increase in epithelial thickness (Marigomez et al., 1990c).

MET values are indicative of digestive epithelium activity (Lowe et al., 1981; Morton, 1983) and of the pathological damage caused by environmental irritants (Lowe et al., 1981; Couch, 1984; Tripp et al., 1984; Marigomez et al., 1986b; Recio et al., 1988). Environmental stress indices the formation of atrophic epithelia (Couch, 1984). These are similar in thickness to R type tubules resulting in a general reduction in MET of the tubule (Marigomez et al.,1990c). In the present study, under histological observation, atrophy of tubules was observed in the digestive gland of animals exposed to copper and lead. This atrophy of tubules lead to reduction in height of digestive cells (Tripp et al., 1984).

Reduction in MET was reported for *L.littorea* after a long exposure to high concentration of cadmium while the MLR values increased (Vega et al., 1989). Similar reduction in thickness of digetive tubules in response to environmental stress was reported by several workers (Thompson et al., 1974; Moore et al., 1978a; b; 1980; Lowe et al., 1981; in the mussel *M.edulis* (Thompson et al., 1978) in the mussel *M.californiansis* (Couch, 1984) in the oyster *C. virginica* (Tripp et al.,

1984) in the quahog *M. mercenaria* (Axiak et al., 1988) in the clam *Venus verrucosa* (Marigomez et al., 1986b; Recio et al., 1988) in the slug *Arion ater* (Marigomez et al., 1990).

Marigomez et al. (1990c) reported reduced MET and MTR values in winkles in response to lowering of salinity of the ambient medium. They observed digestive cell shrinkage, which was explained as a compression exerted by the adjoining connective tissue on digestive tubules as a result of increasing its volume due to hyposmotic stress.

Marigomez et al. (1992) reported reduced MET and MET/ MDR values and augmented MLR/MET values in *L.littorea* during the reproductive phase. Considering the above report, in the present study, the animals were selected carefully to avoid such misinterpretation of results. Thinning of digestive epithelium also occurs due to loss of apical cytoplasm (Moore et al., 1978a; b; 1979).

In the present study, tubules of clams exposed to copper, showed a significant increase in the luminal size compared to the control whereas those of lead exposed ones did not show any increase in size of the lumen. Vega et al. (1989) reported an increase in the lumen size in *L.littorea* exposed to cadmium. This increase in lumen size is inherent to the process of epithelial thinning (Couch, 1984). The formation of atrophic tubules is a part of the detoxication mechanism and is a prompt response to the presence of pollutants. Its expression in MET reduction might be masked by some other factors affecting digestive cell volume. Thus, the

inflammatory response (consisting of haemocyte proliferation by connective tissues) is correlated with the increase in digestive cell volume (Marigomez et al., 1989) associated with tubule dialation. The above observation and loss of apical cytoplasm (observed under electronmicroscopy) were observed in the present study. Similar instances were also reported earlier (Moore et al., 1978a; b).

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5.7. ULTRASTRUCTURE

The Environmental Impact Assessment Programe envisages the methods which measure the biological effects (stress indices) of pollutants on the health condition of organisms (Bayne, 1980; Widdows et al., 1981; Bayne et al., 1982; Livingstone, 1982; Moore, 1985). Stress indices have been developed at different biological organisations (Scott & Major, 1972; Abel, 1976; Hrs-Brenko et al., 1977; Widdows, 1978; Bayne et al., 1980; Axiak & George, 1987; Axiak et al., 1988; Vega et al., 1989). In fact, these indices can be considered as biomarkers of toxicity to central metabolic organs such as the digestive gland of mussels. Investigations at the sub cellular level can detect the sign of damage at an early stage before it shifts to the higher, irreversible stage. This involves studies mostly concentrated around the damages caused to various cell organelles like lysosomes, mitochondria, golgi bodies, endoplasmic reticulam and nucleus. In the present study, alterations of different cell organelles were observed.

Marigomez et al. (1990d) reported increased number of dialated

smooth endoplasmic reticulam cisterns in the excretory cells of the renal epithelium of cadmium treated *Littorina littorea*. Similarly, in the present study also, an extensive proliferation of smooth endoplasmic reticulam was observed. This situation is indicative of either an enhanced protein synthesis or a large excretion from excretory cells. Nott et al. (1985) reported proliferation of smooth endoplasmic reticulam in *M. edulis* exposed to PNAH (Polynuclear aromatic hydrocarbons) due to increased protein synthesis. He suggested that the production of an enzyme [NADPH - neotetrazolium (cytochrome P- 450) reductase, a component of the cytochrome P-450 detoxication/toxication system] in response to the entry of the pollutant. This enzyme is associated with the endoplasmic reticulum.

According to Marigomez et al. (1990d), dark mitochondria are mainly involved in substance accumulation and therefore are less energetic. Smith & Ord (1983) opined that either an increased excretory activity or enhancement of protein synthesis cause the condensation of mitochondria. In the present study, the number of dark and condensed mitochondria was found to be increased in metal exposed animals. This affects the normal function, i.e., the oxidative phosphorylation taking place in the mitochondria due to exposure to divalent cations (Crespo & Sala, 1986).

According to Moore (1985), the earliest detectable alterations are associated with lysosomes, a particular class of sub-cellular organelles mainly involved in the intracellular digestion of food, cellular defence mechanisms, protein and organelles turnover and regulation of secretory process.

Lysosomes are known to play a major role in the metabolism of heavy metals (Sternlieb & Goldfischer, 1976). Metal sequestration was observed in the lysosomes of mussel tissues by several authors (George et al., 1976; 1978; Moore & Lowe, 1977; Schulz-Baldes, 1978; Harrison & Berger, 1982; Viarengo et al., 1987; Etxeberria et al., 1994). Enlargement, swelling, loss of integrity and destabilisation of lysosomal membrane are the stress indices reported (Lowe et al., 1981; Moore, 1980). All the above disturbances were noticed in the lysosomes of the treated animals in the present study.

Metals are known to be associated with metal binding proteins which may enter lysosomes and follow the catabolic pathway as any other cellular protein. However, excessive concentrations of metals can cause alterations of structure, permeability and integrity of lysosomal membranes when the storage capacity is overloaded (Moore, 1980). Accordingly, Viarengo et al. (1981) indicated that copper may act as lysosomal destabiliser in digestive cells of *M. galloprivincialis*. Harrison & Berger (1982) observed a decrease in the latency of lysosomal hexosaminidase in the digestive cells of *M. edulis* at increasing copper doses.

The formation of enlarged lysosomes by the fusion with primary lysosomes has been reported earlier and is considered as a part of cellular autophagy (Lowe et al., 1981; Moore et al., 1987; Lowe, 1988). In certain cases, enlarged lysosomes are formed by the fusion of small organelles and this has been previously described in *M. edulis* after long term exposure to oil derived hydrocarbons (Lowe et al., 1981) and in

winkle *L. littorea* exposed to sublethal concentrations of cadmium (Marigomez et al., 1989). This condition of fusion of organelles with lysosomes is reflected by the multilayered nature of the lysosomal membrane (Nott et al., 1985). This feature of the lysosome was also noticed in the present study. Swelling of lysosomes, which is considered to be a step previous to the lysosomal fusion, was demonstrated to occur in a four day study concerning the toxic effects of alpha-naphthol on *L. littorea* (Cajaraville et al., 1988).

The tertiary or residual bodies are found in bivalves as a result of metal accumulation and are later excreted (George et al., 1976; Viarengo et al., 1981; Viarengo, 1989). This type of metal rich tertiary lysosomes could be seen in the present exposure study.

6. SUMMARY

Pollution studies with special reference to heavy metal contamination is gaining momentum in the present world of industrialisation. Monitoring of metal contamination in the coastal waters is of much value while considering their non-biodegredable nature. These can cause many deleterious effects on the flora and fauna of the aquatic environment which in turn can result in serious public health hazards. Bivalves were accepted world wide as monitoring or sentinel organisms because of their ability to accumulate metals from the surrounding medium to a much higher level.

As the data presented and the results discussed shows that this study was a comprehensive investigation to assess the level of metal pollution and the related environmental Parameters in the Cochin estuarine system (including Vembanad Lake) and also to evaluate the effect of these metals on a commercially important biological resource, *V. cyprinoides.*

All important aspects such as whole body levels, accumulation in the digestive glands and gills, toxicity (acute and sublethal) as well as histology and electron microscopy were also conducted to study the cellular and sub-cellular level changes caused by metals such as copper, cadmium and lead. Modern methods such as planimetry and image analysis techniques were also made use of in interpreting the results.

Findings of the present investigation are summarised below

1. Accumulation of metals in the whole soft tissue of clams showed a monsoon and post-monsoon maxima and pre-monsoon minima for copper. While the cadmium values were slightly higher during the pre-monsoon season. The lead values were very low.

2. Analysis of metals in the digestive gland of clams revealed that copper concentration was high during monsoon and post-monsoon season. Highest concentration of copper was observed in the digestive glands of clams collected from station 5. Low values were observed for cadmium and lead.

3. The seasonal study conducted on four stations recorded highest value for copper and lead in the sediment and clam samples at station 8. Cadmium values were low in all the four stations.

4. Study conducted on the dependence of metal accumulation on increase of clam size revealed that the level of accumulation increased with increase in size.

5. The stations selected for the study did not reveal any significant increase or decrease in any of the hydrological parameters studied. But a slight increase could be observed in

nutrients like phosphate and nitrate.

6. Analysis of metals (copper, cadmium and lead) in the sediment collected fortnightly from the stations did not show a seasonal trend in accumulation. Mean concentrations of copper, cadmium and lead were high at station 5.

7. Acute toxicity tests conducted for copper and lead revealed that copper was the most toxic metal than lead and is harmful to the organism even at a low concentration of 1 ppm.

8. Sublethal tests conducted for copper and lead showed that the digestive glands accumulated maximum level of copper whereas the gill accumulated maximum concentration of lead.

9. Histology of digestive gland and gills of metal exposed clams showed various structural alterations including destruction of epi thelial cells. This was further manifested by the reduced MET values observed in the planimetric study. Thinning of tubules, loss of cilia in the digestive diverticula and wandering of haemocytes were other important observations. Gills of copper exposed clams showed breakage at the interfilamentar junctions, shrinkage of lamellae. Lead exposed clams showed flattening and complete destruction of lamellae in the gills.

10. Histological alterations were further evidenced by the significant increase in the planimetric parameters like MLR, MLR/MET values of copper exposed clams. Though an increase was observed in parameters like MLR and MLR/MET values for lead exposed clams, it was not of much significance.

11. Electron microscopy of digestive glands of copper and lead exposed clams revealed an increased number of lysosomes with consequent increase in their size. There was an increase in the number of basophilic cells in both groups of clams. Considerable destruction of nucleus, vacoulation of cytoplasm, rupture in the basal lamina were noticed in copper exposed clams. Proliferation of smooth endoplasmic reticulam was observed in lead exposed clams.

12. Considering the commercial and Socio-economic importance of the species studied, it is suggested that more studies have to be conducted to fully understand the biochemical and nutritional composition of the clam tissue which is used for human consumption in the light of the present results of metal level concentrations.

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